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Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop: October 4-7, 1992, Edmonton, Alberta

Comptes rendus dix-neuvième atelier annuel sur la toxicité aquatique: du 4-7 octobre 1992, Edmonton, Alberta

Editors/Éditeurs

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1942

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PREFACE/PREFACE

The 19th Annual Aquatic Toxicity Workshop was held at the International Hilton Hotel in Edmonton, Alberta, Canada on October 4-7, 1992. The Workshop included 5 plenary presentations, 80 platform papers, 20 poster sessions and 3 workshops. Total attendance was 269.

The 19th Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to anthropogenic discharges into Northern aquatic environment, new toxicity testing methods, and pathways and fate of contaminants in the Aquatic Environment. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are publicised with the support of the Department of Fisheries and Oceans.

Le 19^e Atelier annuel sur la toxicité a eu lieu à International Hilton Hotel, Edmonton, Alberta du 4 au 7 octobre 1992. L'atelier a donné lieu à: 5 séances plénières, 80 exposés, 20 communications par affichage et 3 ateliers. Un total de 269 personnes ont assisté à l'Atelier.

Le 19^e atelier annuel sur la toxicité aquatique a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique, l'écotoxicologie, les émissions anthropogéniques dans les eaux du Nord, les nouvelles méthodes de contrôle de substances toxiques, les parcours et le sort des contaminants présents dans l'environnement aquatique. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS/REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. A Table of Contents and a List of Participants are also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. Papers were taken from the author's submissions of 90 mm discs. They were reviewed, edited and reprinted. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ce volume renferme les comptes rendus, les textes intégraux ou les résumés de toutes les communications présentées à l'Atelier. Une table des matières et une liste des participants sont aussi incluses. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. Les auteurs ont soumis leurs articles sur des disques de 90 mm. Les textes furent révisés, corrigés et réimprimés. Prière d'adresser toute remarque concernant les textes aux auteurs-mêmes. Toutes les déclarations et les opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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THE TSCA INTERAGENCY TESTING COMMITTEE - WHAT, WHEN, WHO AND WHY?

John D. Walker, TSCA Interagency Testing Committee, USA EPA, Washington, DC, USA.

What is the TSCA Interagency Testing Committee (ITC)? The ITC is an Advisory Committee to the Administrator of the USA Environmental Protection Agency. When was the ITC created? The ITC was created by the USA Congress in 1976 under Section 4(e) of the Toxics Substances Control Act. Who is on the ITC? Statutory Members were appointed from 8 USA government organizations. Ten USA government organizations with experience in chemical testing also participate on the ITC as Liaison Members. Why was the ITC created? Congress created the ITC to screen and select chemicals and chemical groups for priority health effects, chemical fate, and ecological effects testing consideration by the EPA Administrator and to facilitate coordination of chemical testing among the Statutory and Liaison organizations represented on the ITC. How many chemicals have been selected for testing? The ITC has selected 133 chemicals and 41 chemical groups for testing. How many tests has the ITC recommended? About 5,112. How are the data used? To increase the knowledge of chemical persistence and toxicity, to modify industry's Material Safety Data Sheets and to modify regulatory criteria such as EPA's National Effluent Discharge Pollution Elimination System permits and EPA's Reference Concentrations or Reference Doses.

THE FORWARD END OF THE HOG. D.W. Stokes, Alberta Environment, Edmonton, AB, (403) 427-6102.

The Chairman of the Organizing Committee, Earle Baddaloo, asked that I address two questions this morning:

1. DOES THE PUBLIC BELIEVE SCIENTISTS WHEN THEY SPEAK PUBLICLY?
2 WHAT DOES THE PUBLIC KNOW ABOUT AQUATIC TOXICITY?

To the first question: does the public believe scientists when they speak publicly, the answer is yes. Scientists' credibility in the eyes of the public has already been established. For example, in survey research undertaken in Canada and the USA on credibility and trust associated with radiation leaks, researchers asked: who is the most credible, the next most credible, and the least credible in terms of risk communication:

Most credible	-	university professors, health scientists, and employees;
Next most credible	-	media and environmental groups;
Least credible	-	industry and government.

Since scientists have public credibility, it is then very important how you communicate with the public and it should not be taken lightly. Effective communication is difficult because of:

- Complex, confused messages;
- Lack of trust and credibility of information sources;
- Media distortions;
- Public misperception and unrealistic expectations; etc.

For you, the scientist, communicating effectively is a matter of maintaining or enhancing credibility communicating with empathy and clarity in a language the public understands. Why "with empathy"? Scientific facts do not carry a meaningful message for non-scientists or the public. Scientists have to nurture trust and credibility by the way in which they present themselves and speak to the public. The factors to nurture trust and credibility are:

empathy - caring	50%;
competence - expertise	10-20%;
honesty - openness	10-20%;
dedication - commitment	10-20%.

Research has shown that empathy - caring accounts for 50% of trust and credibility in the eyes of the public. Competence - expertise, honesty - openness, and dedication - commitment, each account for 10 to 20%. So empathy - caring is the biggest factor in nurturing trust and credibility with the public.

For you to be empathic when you are speaking to the public, you have to know where they are coming from; what their concerns are. One way to find out what the publics' concerns are is to conduct a survey. If the survey is conducted with appropriate methodology, you can obtain consistent results which are representative of the publics you survey with known statistical precision. Other methods, such as focus groups, are available and cheaper, but how representative they are of the general public is not known. One recent survey of community concerns found: (1) health; (2) safety; (3) environment; (4) aesthetic issues; (5) equity; (6) cultural/symbolic issues; (7) legal/statutory issues; and (8) public policy issues.

In addition to finding out what the publics' concerns are, you will find that sometimes that concern is a perception. You have to accept the fact that perception is a public reality. In your opinion, it may not be correct or accurate, but you have to address that perception as if it is real.

Suppose you have to speak to a community for which health issues are a major concern, there is a gender difference between male and female spokespersons for matters of health communication:

Male versus female spokespersons for health communication trust & credibility factors:

Trust & Credibility Factors	Male	Female
- Empathy/caring		50%
- Competence/expertise	10-20%	
- Honesty/openness		10-20%
- Dedication/commitment		10-20%
	20%	80%

The female is worth 80% just by being there. She can build up her competence/expertise by using third party credibility. In similar circumstances, a man carries 20% from competence - expertise. As scientists, you would have dedication - commitment as well, so male scientists begin with 40%. That is why you have to focus on empathy - caring and honesty - openness if you are to build credibility with the public.

People make a judgement about empathy and caring in 30 seconds. When you are listening to someone, you know they care by the way they speak, their tone of voice, and the kinds of things they say. If you want to ensure that your message is getting across, you have to be aware of people's attention spans. Fifteen minutes is usually tops. But no matter what the length of your presentation, empathy/caring has to come right up front and take about one quarter of the time allotted, whether it is 15 minutes at a public meeting or 30 seconds on the radio, if it is to be effective.

Honesty and openness are demonstrated mostly by non-verbal communication. You have to be really honest and sincere when you are speaking in public. It is almost instinctive how you know you can trust someone.

But I want to reassure you. We are not speaking about manipulating the public but about being sincere in your dealings with the public and the media and emphasizing those aspects of communication which the public finds more meaningful.

If you want to lose trust and credibility, there are lots of ways it can be done. Here, for example, are ten ways to lose trust and credibility:

1. don't involve people in decisions;
2. hold onto information;
3. ignore peoples' feelings;
4. don't follow-up;
5. if you make a mistake, deny it;
6. if you don't know the answer, fake it;
7. don't speak plain language;
8. be a bureaucrat;
9. delay talking to other organizations; and
10. send your introverted scientists.

Why "with clarity in a language the public understands?"

What is the level of public understanding? Do people in different parts of Edmonton have different levels of understanding? Do people in different parts of Canada have different levels of understanding?

For example, in a Gallup Poll carried out July 1, 1991, on a series of general knowledge questions about Canada, different regions across Canada, excluding Quebec, showed a range of differences between 8% and 31% in the proportions getting correct answers on eight questions. When Quebec is included, the range of differences increases to between 14% and 57% across the regions. So there is evidence of regional differences in public understanding and knowledge across Canada. For the publics you have to address, the question of how and what they understand has to be researched.

Where the level of public understanding was researched at Columbia University, it was found that the general public in Canada and the USA had the level of understanding of a Grade XII student. For example, when an official of the Environmental Protection Agency stated on television that "The substance may leach from this site and migrate into a plume," 40% of the Grade XII students thought he was speaking about "carnivorous worms." When an official of a State environmental agency was speaking about laws which contained apparent contradictions, he said "That's the paradox of the statute." The Grade XII students thought he was speaking about a statute of a pair of ducks.

Back to the first question, the answer is, yes, publics do believe scientists, but when you are speaking to the public, even through the media, if you do not take pains to ensure that you speak with empathy and with clarity in plain language, your credibility will be lost.

One example of the enormous effort required to re-establish communication and credibility with the public is the extent to which the Chemical Manufacturers Association has gone since the incident at Bhopal with their Community Awareness program.

Now to question 2: WHAT DOES THE PUBLIC KNOW ABOUT AQUATIC TOXICITY?

Let us take a look at the Workshop brochure with the understanding of a Grade XII student, bearing in mind that a Grade XII student may be able to use a dictionary but would go to any lengths to avoid it. Let's see now, "Aquatic Toxicity." Aquatic? Isn't it a toothpaste? Aquatic sounds wet; it has something to do with water. Toxic is poison. So aquatic toxicity must mean either wet poison or poisonous water. If the student does not look it up in the dictionary, the meaning is not all that clear. Looking on the front page of the brochure, there is a symbol of three drops of water and against each drop there is a title. The first is "Anthropogenic discharges into northern aquatic environment." Without too much hesitation, the Grade XII student will readily identify that it means "insects polluting northern rivers and lakes with the genes." To obtain that understanding it was necessary to change only one letter in the first word. The next title is even easier. It says: "New toxicity testing methods: approaches and evaluation." Toxicity is poisonous. Alcohol is poisonous. Alcohol testing methods is simply "research into breathalysers." We know the police are always trying to find a better way to test us for it.

The third title is difficult. We have to look up the words in a dictionary. It says "Pathways and fate of contaminants in the aquatic environment." The first meaning of "pathways" is "a place where you walk or a direction or course". The meaning of "fate" is "final outcome or disaster." So, it seems to have something to do with a direction to disaster. "Contaminant" is an "intrusion of dirt or foulness from an outside source." Without looking it up, "aquatic environment" is "rivers and lakes." So far, we have a meaning for "pathways and fate of contaminants in the aquatic environment" as "a direction to disaster in rivers and lakes from dirt or foulness from outside." Is that what was intended? As a Grade XII student though, I am still not sure that I understand so I have to look up my dictionary some more. Another meaning of "pathways" is the "sequence of enzyme catalized reactions by which an energy-yielding substance is utilized by protoplasm." Well, without going to the dictionary, as a Grade XII student I recognize an "energy-yielding substance." That's my sister's husband when he gets mad and starts breaking up the house with a baseball bat. He is yielding lots of energy. Does that make sense with this meaning of "pathways?" "Sequence" is "a succession of repetitions." That's okay. He does it quite often. "Enzyme" is "a complex protein produced by a living cell." When he's mad he's producing lots of protein. "Catalized reactions" is "reactions between two or more persons precipitated by a separate agent and especially by one that is essentially unaltered by the reaction." That fits my sister's husband perfectly; he's never affected by what he does. "Protoplasm" is "the organized colloidal complex of organic and inorganic substances." When I looked up "colloidal" and found that it meant a gelatinous substance found in diseased tissues we were all the way back to contaminants. So, to this Grade XII student making some use of a dictionary, "Pathways and fate of contaminants in the aquatic environment" is "a study of how does the outside dirt and foulness that make my sister's husband mad enough to break up the house with a baseball bat without his being affected, affect rivers and lakes."

These are times when you need public support for your research if you are to receive any funding at all. To get public support you have to build your credibility especially by enhancing your communication skills. That means focusing on empathy - caring and honesty - openness in delivering your message. It especially means: do not leave so much of what you mean to say open to public (or Grade XII) interpretation. Be very clear and precise about what you mean in plain language.

If what I have said sounds far-fetched or unbelievable, consider the title of this talk "The Forward End of the Hog . . ." When you first heard that, what came to mind? If it was some picture of the front end of a hog, "Pigs," it is quite understandable because you have been deliberately misled. Please understand, I am in no way inferring that I was the front end of the hog, or that you were, or the rear end either. Placed in its proper context, the complete quotation is: "The forward end of the hog has to match up with the rebate in the stem, so that the twisted garboard strake drops into a properly faired rebate." Percy W. Blandford. 1971 (1966) Build Your Own Boat. London: Stanley Paul. p.68. It means "Boatbuilding overhead." There is the hog. It is the board that is fitted above the keel in a clinker stem dinghy. There is the garboard stroke. There is the rebate. "Properly faired" in boatbuilding means to make smooth and regular. If you can properly fair your communications with the publics, hopefully you will retain your credibility and receive public support for your research.

NOTE: Research at Columbia University is conducted by Dr. Vincent T. Covello, Professor and Director for Risk Communications.

THE TOLERANCE OF ARCTIC MARINE INVERTEBRATES TO ZINC AND LEAD AND IMPLICATIONS FOR ARCTIC CHEMICAL DISCHARGES.

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ABSTRACT

Epontic and benthic amphipods (*Onisimus litoralis*, *Onisimus* juveniles, *Gammarus setosus*, *Anonyx nugax* and *A. makarovi*) and a pelagic mysid (*Mysis oculatus*) were collected under-ice at Little Cornwallis Island, North West Territories, Canada. They were exposed, on site, to dissolved zinc and lead to determine lethal concentrations (LC50s). Incidental information was obtained on salinity and temperature tolerance. Subsequent testing of a temperate amphipod exposed to the two metals provided comparative data to augment a literature review. The arctic invertebrates were surprisingly insensitive under all test conditions. The implications for arctic developments, particularly those involving near-shore, point-source discharges, are discussed and recommendations are made for effectively expanding a presently depauperate arctic marine toxicity data base.

INTRODUCTION

Eight countries (Canada, Denmark, Finland, Iceland, Norway, Sweden, U.S.S.R. and USA) signed the Declaration on the Protection of the Arctic Environment (Rovaniemi, Finland, June 14, 1991). This document expressed particular concern for the effects of chemical pollution, including the effects of heavy metals, and for the assessment of potential impacts of development activities. However, remarks made by Dr. Fred Roots (Chairman, International Arctic Science Committee) at this Ministerial Conference indicated that much information necessary to the protection of arctic regions is unknown, in particular their sensitivity to change.

The present study provides information which will assist in the protection of arctic regions, specifically the short-term toxicity of two metals, lead and zinc, to near-shore arctic marine invertebrates. Although the literature contains extensive information on bioaccumulation of contaminants by arctic fauna and toxicity tests have been conducted with oil (e.g., Percy, 1976, 1977; Riebel and Percy, 1990), we were unable to find evidence for any previous metals toxicity testing with arctic marine fauna. If there truly are no such previous data, then this study is unique, but hopefully will not remain so. Recommendations for effectively expanding the apparently depauperate (for metals and most other chemicals) arctic marine toxicity data base are provided, and the general and specific implications of this study for near-shore, point-source discharges to the Arctic are discussed.

This study was commissioned by Cominco Ltd.'s Polaris Mine (located on the southern tip of Little Cornwallis Island at 75°23'N, 96°55'W, the most northerly based mineral mine in the world; Figures 1 and 2). The mine produces lead and zinc, and discharges its tailings at depth in meromictic Garrow Lake (Dickman and Ouellet, 1987). Discharge of surficial water from Garrow Lake is to the adjacent arctic marine environment of Garrow Bay. The mine undertook this study as part of their environmental management strategy.

METHODS

Field Experiments

Field studies were conducted in June 1991. At the time of testing, the ice on Garrow Lake and Garrow Bay was approximately 2 m thick and the ambient air temperature was approximately 3 to 9°C. A temporary field laboratory was set up in a shelter on the shore of Garrow Bay, just east of the mouth of Garrow Creek. The shelter was equipped with two large waterbaths which held the toxicity test containers. Surface water from Garrow Bay was periodically pumped through the waterbaths by means of a peristaltic pump, and blocks of ice were also used to provide temperature control. The test containers were new, plastic ice cube trays covered with 2 mm mesh screens. The ice cube trays were soaked in Garrow Bay water for approximately 12 h and rinsed twice before the toxicity tests were initiated.

Test Organism Collection

Test organism collection began on June 12, 1991. Minnow traps covered with 2 mm mesh screening material were used to collect test organisms from Garrow Bay. The traps, baited with catfood and weighted with small rocks, were set approximately 100 m from the shore, in holes drilled with an ice auger and in a 0.5 m wide "lead", and left overnight. Several large amphipods were observed swimming near the surface of this lead. Initially the traps were set just below the bottom of the ice, to collect any epontic organisms. The following day, several types (different species or age-classes) of amphipods were recovered from the traps. The animals were sorted and held for several hours in surface water collected from Garrow Bay, to ensure that the animals would survive handling. Because the surface water used to hold these animals had a very low salinity (approximately 2 ppt), they were not used for the actual toxicity tests, to eliminate concerns about stress. Subsequent collection of Garrow Bay seawater for holding and testing involved deeper, more saline waters. The minnow traps were re-set on June 13, 1991 just below the ice and on the bottom to collect benthic species. When the traps were recovered several hours later, four types of amphipods (three epontic and one benthic) were collected. Samples of each type of amphipod were preserved in 5% buffered formalin for taxonomic identification.

Taxonomic analysis (by Dr. E. Bousfield, Royal British Columbia Museum) indicated that four and possibly five different species had been collected and tested. *Onisimus litoralis* were approximately 1 cm long and pink or golden in colour with a red stripe and bright red eyes. Small, golden brown amphipods, 3 to 5 mm long, were identified as *Onisimus* sp., possibly juvenile *O. litoralis*. *Gammarus setotus* were grey-gold and black and were "feathery" in appearance. Most of these animals were 1 to 2 cm long, although a few were smaller (0.5 cm long). The benthic amphipods were the largest animals, approximately 2 to 3 cm long, and consisted of two species of the same genus. The larger animals (*Anonyx nugax*) were bright pink or orange and the smaller animals (*A. makarovi*) were a golden colour.

The organisms were sorted and placed in 1-L plastic jars containing Garrow Bay seawater (33 ppt) for approximately 7 h prior to initiating the first round of toxicity tests with the pure metal solutions.

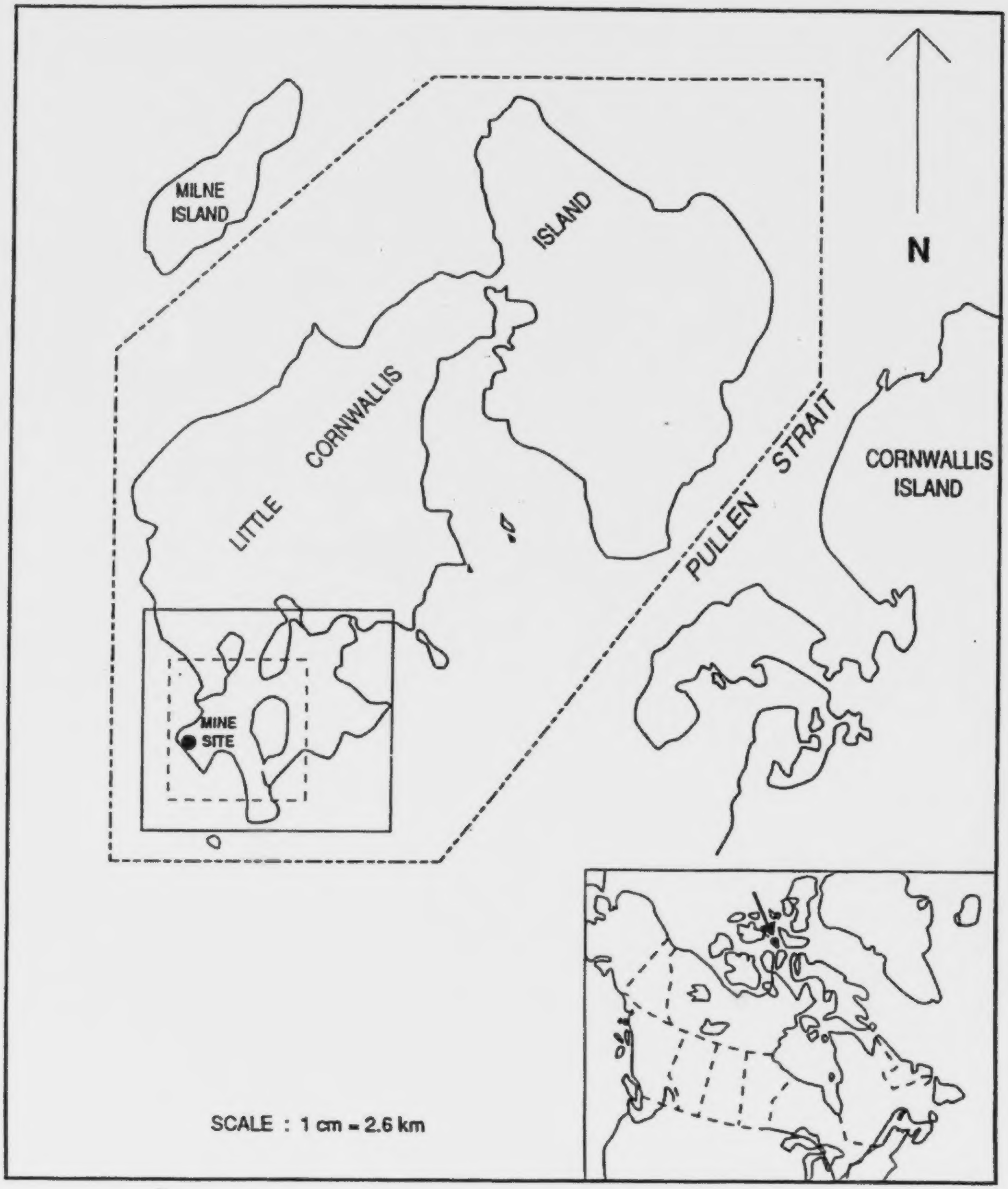
MILNE ISLAND
LITTLE CORNWALLIS ISLAND
PULLEN STRAIT
CORNWALLIS ISLAND
MINE SITE
N
SCALE : 1 cm = 2.6 km

Figure 1. Location of Polaris Mine, Little Cornwallis Island, N.W.T.

On one occasion, plankton tows were conducted at this site at various depths. A large number of small mysids (*Mysis oculatus*) were recovered. A sample was collected for taxonomic identification and a 24-h toxicity test was conducted to measure their response to zinc solutions. Longer testing was not possible because field time was almost exhausted when these animals were collected.

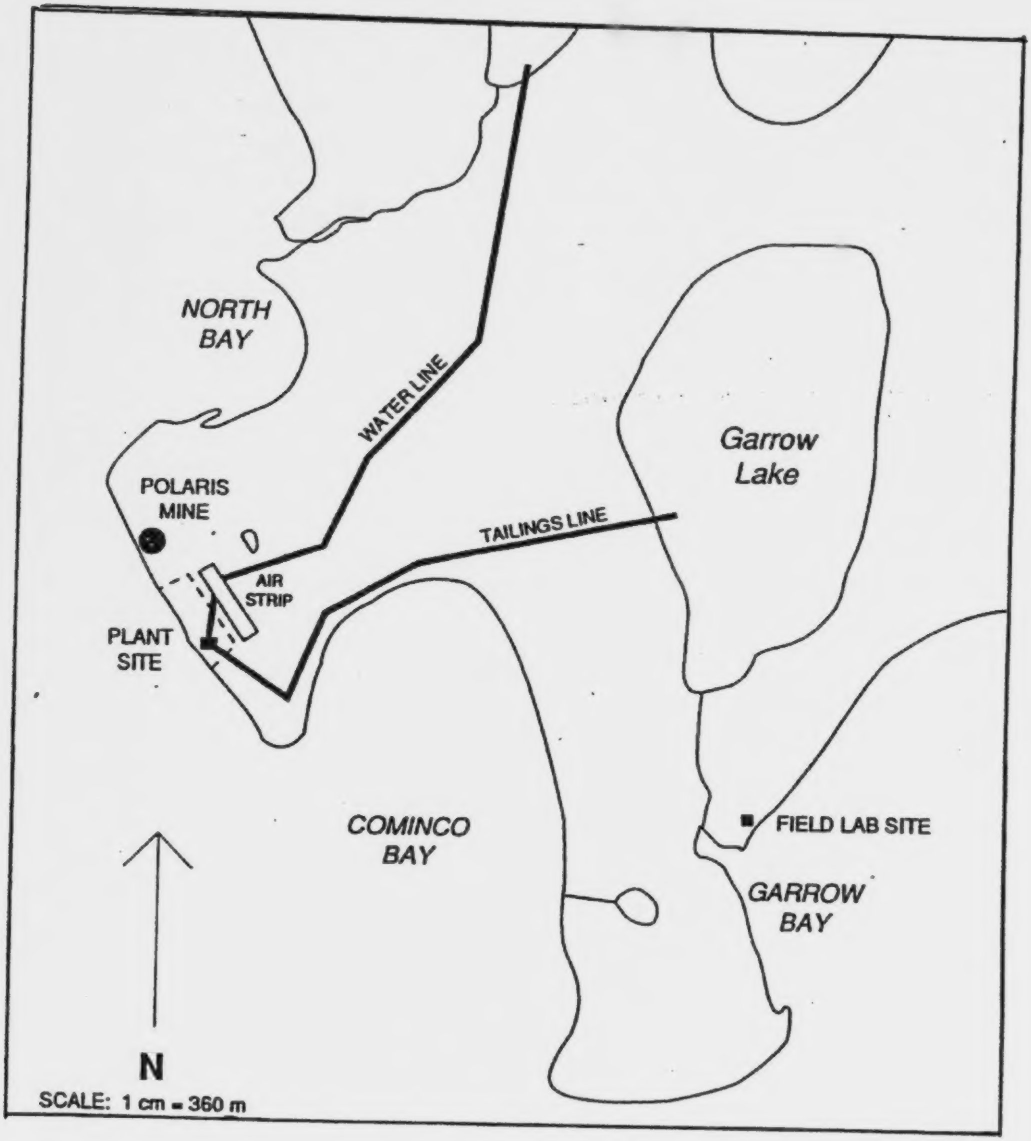
NORTH BAY
WATER LINE
POLARIS MINE
TAILINGS LINE
AIR STRIP
PLANT SITE
Garrow Lake
COMINCO BAY
FIELD LAB SITE
GARROW BAY
N
SCALE: 1 cm = 360 m

Figure 2. Details of Polaris Mine Site.

Collection and Handling of Water Samples

Water samples were collected from Garrow Bay 5 m below the ice, at the amphipod collection location, using a vacuum intercept pumping technique. A weighted (polyethylene coated lead) polyethylene 1.25 mm ID line was lowered through a hole in the ice, flushed with several tubing volumes of water and then used to fill 20-L acid leached glass carboys. Sufficient water was collected to perform the field and laboratory experiments and as well as to allow chemical analyses for background zinc and lead concentrations. This water had a salinity of 33 ppt and was used as the dilution and control water for the field and laboratory experiments. Surface water from Garrow Bay (2 ppt salinity) was collected in buckets and used to rinse test containers and equipment. Water from Frustration Lake (the mine's freshwater supply) was collected from a tap (after 1 min of flushing) in the mine's accommodation building. The salinity of this water was then adjusted to 7 ppt and 55 ppt by adding Bio-Marine aquarium salt mix. All carboys were sealed, labelled and placed in styrofoam overpacks to prevent breakage. The carboys were stored outside the shelter (ambient air temperature 3 to 9°C) until they were shipped to the laboratory on June 19, 1991 (Garrow Bay water was collected on June 13 and 18). Upon arrival at the laboratory, the samples were stored at 4°C in the dark until used for testing.

Preparation of Metal Test Solutions

Pure solutions of individual metals were prepared by mixing measured amounts of zinc and lead with Garrow Bay seawater. One litre of each test solution concentration was prepared. The sources of zinc and lead were 1,000 mg/L for AA standard solutions (Fisher Scientific: Zinc - Cat. No. SZ13-500, Lot No. 904275-24; Lead - Cat. No. SL21-500, Lot No. 900222-24). All glassware used to prepare the test solutions was rinsed in 10% nitric acid (HNO_3) and then rinsed five times in Garrow Bay water prior to use. The required amount of reference solution was dispensed to a graduated cylinder using an Eppendorf micropipettor, with a disposable tip. The volume was then made up to 1 L with Garrow Bay seawater and the solution was transferred to a labelled 1-L polyethylene bottle. Six zinc solutions (0.1, 0.56, 1.0, 3.2, 5.6 and 10.0 mg/L) and six lead solutions (0.1, 0.56, 1.0, 1.8, 3.2 and 5.6 mg/L) were prepared in this manner. Because the reference solutions were acidic, the pH of each test solution required adjustment. A 1N NaOH solution was prepared by dissolving NaOH in Garrow Bay seawater. This solution was used to adjust the pH of each solution to between 7.9 and 8.0, to match that of Garrow Bay seawater. The unused portions of each test solution were held for chemical analysis to determine the actual concentrations of zinc and lead present.

Toxicity Testing

Toxicity testing with the zinc solutions began on June 13, 1991. Tests were conducted under continuous light (ambient) at a temperature of 5 ± 2°C. The test duration was 96 h. For each treatment there were 10 replicates, with one amphipod each. Garrow Bay seawater was used as the negative (clean) control. The test containers were prepared by dispensing the zinc solutions to the individual compartments in the ice cube trays. For the *Onisimus* juveniles and *O. litoralis*, all six zinc concentrations, plus a Garrow Bay control, were prepared. For *G. setotus*, only four concentrations (0.1, 0.56, 1.0 and 3.2 mg/L Zn) were prepared because of a shortage of animals; the Garrow Bay control was also omitted. Testing of *Anonyx* involved all six zinc concentrations, but omitted the control because of a shortage of animals. Once all the test solutions were dispensed, each chamber was seeded with one amphipod. Amphipods were transferred using a plastic spoon, taking care to minimize the amount of water added with each animal. Since taxonomic identifications had not been confirmed, efforts were made to expose equal numbers of *A. nugax* and *A. makarovi* to each treatment. After seeding, all the chambers were checked again and any amphipods trapped on the water surface were gently re-submerged. Screens were placed over each ice cube tray to prevent movement from one chamber to another.

The lead solutions were tested on June 14, 1991 using the same procedures described above. There were insufficient numbers of *G. setotus* and *Anonyx* for testing. For both types of *Onisimus*, the full series of six lead concentrations, plus controls, was tested.

Tolerance tests with salinity-adjusted Frustration Lake water were initiated on June 14, 1991. Eight individual *Onisimus* juveniles and eight *O. litoralis* were exposed to 7 ppt and 46 ppt for 96-h. A shortage of animals precluded other testing.

A 24-h mysid toxicity test was initiated on June 18, 1991. Ten *Mysis oculatus* were exposed to each of three concentrations of zinc (10.0, 25.0 and 50.0 mg/L), plus a control.

The test containers were checked at least three times daily; mortality counts were made every 24 h. Amphipods trapped at the air/water interface were gently re-submerged. On several occasions during incidental testing, *Anonyx* individuals crawled into adjacent chambers and had to be returned to the proper location. Water was circulated through the water baths periodically to maintain temperature control. The pH and salinity of the test solutions were monitored at the start and end of the tests. Dissolved oxygen levels could not be measured due to an equipment malfunction; the water samples were assumed to be saturated at the low temperatures at which they were collected. Salinity was measured with a hand-held refractometer (calibrated in freshwater), and pH was measured with a portable pH meter (calibrated to standard buffer solutions). After 96 h, survival in each treatment was recorded for subsequent determination of 96-h LC50 values.

Incidental Testing

During the course of the field experiments, incidental observations were made as follows. The short-term tolerance of the amphipods to increased temperatures was assessed when animals used for a display at the mine were stored in an office for 48 h. The test containers were initially held on ice and the ice was replaced after 24 h but the temperature rose to >15°C. A 72-h salinity tolerance test was conducted with both types of *Onisimus*. Ten animals of each species were exposed to Garrow Bay surface water (at 2 ppt salinity) and to a salinity of 55 ppt obtained by adding aquarium salts to the surface water. Several of the *Anonyx* individuals that crawled into other chambers entered ones which did not contain test solutions. These animals were out of water for several hours but did not appear to show any short-term effects.

Laboratory Experiments

The zinc and lead reference solutions and unused water samples from Garrow Lake and Garrow Bay were returned to the EVS Consultants laboratory for further testing with a standard temperate organism, a marine infaunal amphipod (*Rhepoxynius abronius*). Test solutions were prepared with Garrow Bay seawater, as described for the field experiments. An additional negative control was tested. Clean seawater from Burrard Inlet, Vancouver, BC (collected at a depth of 12 m) was passed through a sand filter, a 0.5 - 1 µm Cuno filter and an ultraviolet sterilizing apparatus. This seawater was aerated vigorously prior to use and was used within 2 d of collection. The toxicity tests were conducted in constant environment chambers with the required test temperature and photoperiod.

Amphipods were collected subtidally from West Beach, a relatively remote site on Whidbey Island (Washington State), using a bottom trawl. A short haul (20 m) was used to minimize potential damage to animals during collection. Amphipods were maintained in collection site sediment with overlying seawater, transported on ice, and returned to the EVS Consultants laboratory within 8 h of collection.

Following their arrival in the laboratory, the amphipods were kept in holding containers filled with fresh seawater (28 ± 2 ppt salinity) and maintained at 15 ± 1°C under continuous light until used in testing. Seawater in the holding containers was changed every 1 to 2 days and aeration was provided. The amphipods were not fed during acclimation and were held for nine days before testing was initiated. Prior to testing, amphipods were hand sorted from sediments and identifications were confirmed. Damaged, dead or unhealthy individuals were discarded.

Prior to initiating the toxicity tests, fresh solutions of each metal concentration were prepared. The procedures were the same as those used for the field experiments. One additional zinc concentration (0.32 mg/L) was added. The pH of each solution was adjusted with 1N NaOH and unused samples were archived for chemical analysis. Salinity tolerance was tested as for the arctic amphipods.

The negative (clean) controls consisted of Garrow Bay seawater and Burrard Inlet seawater. In addition, a positive (toxic) control was conducted using the standard reference toxicant, cadmium chloride ($CdCl_2$). A series of cadmium concentrations (no sediment) was tested to determine a 96-h LC50 value to measure the health and sensitivity of the amphipods.

The amphipod bioassays were initiated on June 26, 1991 and allowed to proceed for 96 h. The tests were conducted at 15°C under continuous light. The test containers were checked at least once per day to resubmerge any amphipods trapped at the air/water interface, and mortality checks were performed every 24 h. Water quality parameters (pH, temperature, salinity, dissolved oxygen) were measured at the start and end of testing. Temperature was monitored daily. After 96 h, survival was determined for each treatment. The 96-h LC50 values were determined by data observation or were calculated using the EFFL computer program (IBM/AT Vers. 1.0) as detailed by Stephan (1977). As survival was 70% for the highest concentration of zinc tested, an additional series of tests was performed. Three further concentrations (10, 18 and 32 mg/L), plus a negative control, were tested.

Chemical Analyses

The samples of Garrow Bay seawater, collected for analysis of background levels, and the solutions of zinc and lead prepared for the field bioassays were analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS). A Perkin-Elmer Model 703 atomic absorption spectrophotometer coupled to a Model HGA 500 graphite furnace was used for these measurements. The samples for zinc analysis were first diluted by a minimum factor of 50, while the samples for lead analysis were diluted by a minimum factor of 25. Subsequent higher dilutions were made for more concentrated samples. This dilution allowed the samples to be analyzed by GFAAS and reduced the interference due to the high salt content of the samples. For Garrow Bay seawater, lead and zinc were pre-concentrated from the seawater using a modification of the technique described by Danielson et al. (1978). The zinc and lead solutions prepared for the laboratory bioassays were analyzed in accordance with procedures described in APHA (1989) and USA EPA (1986). These measurements were made using a Perkin Elmer Model 3100 dual beam atomic absorption spectrophotometer (flame mode) equipped with automatic deuterium background correction.

In addition to analyzing the water samples, the ice cube trays used as test containers were tested to determine any contamination. New, unused trays were soaked in Garrow Bay seawater in the laboratory for 96-h (to match the duration of the toxicity tests) and the resulting leachate was analyzed for zinc and lead levels.

RESULTS

Chemical Analyses

Zinc and lead levels in the Garrow Bay seawater were 0.17 µg/L zinc and <0.015 µg/L lead. The results of the analyses performed with the ice cube trays indicated that leachable lead was undetectable (<0.015 µg/L) while the total amount of zinc leached into Garrow Bay seawater was 2.5 µg/L. The nominal and actual concentrations of the zinc and lead solutions prepared for the toxicity tests were fairly similar for the field studies but were more variable for the laboratory studies. Unless noted, the toxicity test results for the metal solutions are presented using actual concentrations.

Field Experiments

The results of the field experiments are summarized in Table 1. Zinc concentrations up to 11.8 mg/L were not acutely toxic to *Onisimus* juveniles or *O. litoralis*. *Anonyx* did show some mortality, with 50% survival at 5.8 mg/L but 100% survival at 11.8 mg/L zinc. Most of the mortalities occurred among the larger animals (*A. nugax*), which were overly crowded in the test chambers; test solutions were cloudy and contained a large amount of fecal material where animals had died. Thus these mortalities appear to be an artifact of testing rather than a real response to zinc. *G. setotus* showed no mortality at 4.9 mg/L (the highest zinc concentration tested with this species). There were no mortalities for either *Onisimus* age category exposed to lead concentrations up to 3.5 mg/L. Mysids (*M. oculatus*) did not show an acute response to 50.0 mg/L zinc (nominal) during the 24-h exposure period.

Water quality parameters measured at the start and end of the zinc and lead tests (and daily for temperature) were in the following ranges: temperature, 3 to 7°C; pH, 7.3 to 8.2; and salinity, 34 to 35 ppt.

Laboratory Experiments

The results of the laboratory experiments are summarized in Table 1. Zinc was not acutely toxic to *R. abronius* at concentrations as high as 28.4 mg/L (70% survival). The 96-h LC50 for lead was 14.1 mg/L. *R. abronius* was sensitive to high and low salinities: 60% mortality at 46 ppt; 100% mortality at 7 ppt.

The 96-h LC50 for the reference toxicant, cadmium chloride, was 0.87 mg/L Cd. This value was within the average range (mean ± 2 SD) of 0.61 ± 0.35 mg/L obtained by this laboratory in previous testing. Water quality parameters measured during zinc and lead testing with *R. abronius* were within the following ranges: temperature, 14 to 15°C; pH, 7.7 to 8.5 ; salinity, 27 to 29 ppt; and dissolved oxygen, 6.9 to 8.5 mg/L.

DISCUSSION

Field and Laboratory Experiments

The arctic invertebrates tested showed a surprisingly high tolerance to zinc and lead. LC50 values were not derived because the authors conducted testing with a preconceived notion (which we suspect is generally prevalent among scientists, administrators and the public) that arctic fauna are particularly sensitive to all toxicants. Accordingly, high enough metals concentrations were not tested.

Table 1. Toxicity test results.

Parameter	Toxicity Data					
	96-h LC50 Arctic Amphipods				24-h LC50 Arctic Mysid *M. oculatus*	96-h LC50 Temperate Amphipod *R. abronius*
	Onisimus juv.	*O. litoralis*	*G. setotus*	*A. nugax* / *A. makorovi*		
Pb (mg/L)	> 3.5[1] (0%)[2]	> 3.5 (0%)	NT[3]	NT	NT	14.1
Zn (mg/L)	>11.8 (10%)	>11.8 (0%)	>4.9 (0%)	>11.8 (50% @ 5.8) (0% @ 11.8)	>50.0 (nominal) (0%)	>28.4 (30%)

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[1] > represents the highest concentration tested. Metals are expressed as actual concentrations, except where noted.

[2] Percent mortality (in parentheses) at highest concentration tested, where no LC50 could be derived.

[3] NT = not tested.

Testing of under-ice amphipods represented "worst case" exposures for discharge of contaminated less saline waters, i.e., Garrow Lake surface waters discharged during the Spring melt would probably accumulate under the sea ice where epontic amphipods congregate. Thus these organisms would be exposed to the highest concentrations of Lake run-off and associated contaminants. Further, amphipods as a group are generally considered to be sensitive to contaminants including heavy metals (Bellan-Santini, 1980; Chapman et al., 1987).

Spring sea ice forms a temporary habitat for algae, amphipods and mysids, which are benthic or pelagic at other times of the year. Studies near Resolute (Pike and Welch, 1990) have shown that over 90% of the under-ice macrofauna in the area of the Polaris Mine comprise four species of amphipods, three of which were tested in this study: *Onisimus litoralis*, *Gammarus setosus* and *Onisimus* juveniles.

Because field experiments did not include high enough zinc and lead concentrations to achieve an LC50, the relative sensitivity of the arctic amphipods compared to the temperate amphipod cannot be established, nor can their absolute sensitivity. However, the arctic species are clearly much more tolerant to salinity extremes than the temperate species and are extremely resistant to temperature extremes. High tolerance of environmental extremes is not unexpected given the range of conditions (near-fresh salinities under-ice to high salinities in the water column) these organisms are exposed to naturally, and has been noted by other investigators. For instance, Shea and Percy (1990) found that *O. litoralis* survived 10-d exposures to salinities of 5 to 55 ppt and that *A. nugax* tolerated salinities of 23 to 45 ppt with little mortality. Percy (1975) and Aarset and Aunaas (1987) have shown similar results with species of arctic amphipods not tested in the present study.

Possible chronic (e.g., growth, reproductive) effects of zinc and lead to the four arctic amphipods and single mysid tested were not assessed. There are no methods available for conducting such tests with arctic species and we could find no evidence that such testing had ever been performed in the Arctic. Surprisingly, we were able to find no evidence that arctic marine fauna had been previously tested for tolerance to toxic contaminants other than oil. Public announcements and notices at the two major 1991 meetings of toxicologists yielded no response: 18th Annual Aquatic Toxicity Workshop, September 30 - October 3, 1991, Ottawa, Ontario (this meeting had a Special International Session on Contaminants in the Arctic); and, 12th Annual Meeting of the Society for Environmental Toxicology and Chemistry, November 3 - 7, 1991, Seattle, Washington (this meeting had a Poster Session on Arctic Ecotoxicology). Literature searches and requests for information from government and other scientists also yielded no information for contaminants other than oil.

In cases where chronic data are unavailable, a "safe" concentration can be approximated by multiplying the 96-h LC50 by a "safety" factor of 0.01. This "worst case" safety factor was developed by the USA National Academy of Sciences (Committee on Water Quality Criteria, 1972) and remains in use (e.g., USA EPA, 1985). If this safety factor is applied to arctic amphipod 96-h LC50 data derived in the present study, protective undiluted concentrations are: Pb, >35 µg/L; Zn, >49 to >118 µg/L. Definitive values are not possible because the amphipods did not die at the highest concentrations tested.

Marine toxicity data for lead and zinc encompass a wide range of concentrations, generally three to four orders of magnitude (100 to 1,000-fold). These ranges, none of which reflect data from arctic organisms, are shown in Table 2 together with published water quality criteria, data on ambient zinc and lead concentrations in Garrow Bay waters, and present study results. Clearly the tested arctic marine invertebrates are not more sensitive to lead and zinc than non-arctic species, and might well have proven to be among the most tolerant species if testing had been conducted to higher concentrations.

However, the fact that the tested species were not particularly sensitive to metals does not indicate they will not be sensitive to other contaminants. For instance, Riebel and Percy (1990) found that one of the organisms we tested, *Mysis oculata*, was among the most sensitive of marine crustaceans to petroleum hydrocarbons. However, little information presently exists regarding either organism-specific or toxicant-specific tolerances.

Table 2. Summary of marine toxicity, water quality criteria, and related data for lead and zinc.

	Metal (µg/L)	
	Lead	Zinc
General Toxicity[1]		
Algae	207 - >5,000	100 - 33,600
Invertebrates	17 - 500,000	60 - 750,000
Fish	7.8 - 180,000	>50 - 85,000
Site-Specific Toxicity[2]		
Acute (measured)	>3,500	>4,900 - >50,000
Chronic (predicted)	>35	49 - >500
USA EPA Water Quality Criteria[3]		
Acute	140	95
Chronic	5.6	86
Ambient in Garrow Bay		
Seakem (1991)	≤0.04	0.06 - 0.19
Present Study	≤0.015	0.17

[1] Data summarized from Dear and Chapman (1992). Lowest concentrations include cellular and subcellular responses which may, or may not, be manifested as an adverse effect to the organism (e.g., inhibition of brain cholinesterase in shiner perch, *Cymatogaster aggregata*).

[2] Present study.

[3] USA EPA - current Environmental Protection Agency Water Quality Criteria.

IMPLICATIONS AND RECOMMENDATIONS

A surprisingly major data gap appears to exist in our understanding of the response of arctic marine ecosystems to anthropogenic insults, specifically the effects (acute and chronic) of almost all chemical contaminants on individual organisms. Previous arctic contaminant research appears to have focused on the phenomenon of bioaccumulation, rather than measuring effects other than for oil. This focus is understandable given logistical and other difficulties involved in working in arctic environments. It is relatively easy to collect organisms for tissue chemical analyses compared to conducting live testing on site and, in the Arctic, live testing tends to be project-specific, i.e., literature exists for oil due to extensive previous oil exploration and development activities. Probably also in many cases where testing has been done, it has been conducted off site using non-arctic but standardized test organisms.

There is a clear, immediate and pressing need to develop arctic-specific bioeffects information for contaminants to assess whether and to what extent developments in the Arctic are of concern. Such information is essential to allow an adequate assessment of any hazards of present and proposed developments which discharge contaminants to the marine environment.

There is also an immediate need to develop standardized test procedures for arctic waters, much as has been done in temperate and tropical waters. In this regard, epontic amphipods appear to be good candidates for study. They are important components of the food chain, feeding on algae and being fed on by commercially and ecologically important fish species (Riebel, 1984; Nicklin, 1991). Riebel and Percy (1990) have previously recommended *Mysis oculata* for toxicity testing based on its sensitivity, vulnerability, availability in near-shore waters and ease of maintenance under laboratory conditions. The same comments apply to epontic amphipods. Collection under-ice is relatively easy as is testing, which can be done with more conventional exposure chambers (e.g., glass 1-L beakers) than ice cube trays. However, ice cube trays are easy to transport, do not readily break, and provide ease of observation when testing is done in the field (e.g., in a tent).

Because there are various communities, industries (e.g., mines, oil platforms), and research stations (e.g., floating ice platforms) in the Arctic, there are many readily available opportunities to conduct research into contaminant effects. Some arctic communities are attempting to attract tourists through marine displays in aquarium facilities (e.g., the community of Resolute Bay, NWT), where testing could be accomplished relatively easily. However, as the present study demonstrates, complicated equipment and dedicated facilities are not necessary. Further, testing does not necessarily require chemical analyses of test solutions as was done in the present study (though such is desirable); testing using nominal concentrations will also provide useful information. Such research can be conducted in isolation or by "piggy-backing" on other projects (e.g., adding personnel or duties to present research stations), and provides unique research opportunities for graduate student theses. Finally, government, consulting and industrial scientists involved in studies of arctic developments would be well advised to conduct focused, site-specific bioeffects testing with resident organisms to answer questions of environmental significance, and to publish these studies.

For instance, this study was funded by Cominco Ltd. to address concerns raised by the Northwest Territories Water Board about the Polaris Mine. The results have major implications to that mine and to other arctic mines (e.g., Nanasivik). In the specific case of the Polaris mine, this study indicated that, because their discharges of lead and zinc (maximum permit concentrations are 0.040 mg/L Pb and 0.300 mg/L Zn) are at least an order of magnitude lower than non acutely toxic concentrations (>3.5 mg/L Pb and >4.9 to >11.8 mg/L Zn) and are diluted prior to reaching the marine environment, mortalities of arctic marine fauna will not result. Mortality is the main factor affecting populations exposed to heavy metals, including lead and zinc (Enserink et al., 1991).

This study also provides information useful for assessing effects of other arctic discharges, in particular seasonal point-source, shore-line discharges. Seasonal discharges are not uncommon in the Arctic due to the freeze and thaw cycle. Similarly, near-shore point-source discharges are not uncommon.

Shore-line discharges initially impact ice-scoured areas where productive aquatic fauna may be conspicuously absent, allowing for dilution to occur. However, "worst case" lack of initial dilution can occur in these areas if discharge occurs before melting of sea ice, when under-ice barriers may concentrate the discharge near-shore so that no effective dilution occurs. Dilution and timing of discharge are important factors in controlling or eliminating any effects on arctic marine fauna, where such control is possible.

Discharges with lower salinity than marine waters will tend to concentrate under ice with concomitant "worst case" exposures to highly productive epontic communities (as is true for oil). The present study tested epontic fauna for this very reason, because Garrow Lake run-off is of low salinity. Such worst case exposures should be avoided even when, as in this study, the epontic fauna prove tolerant. This can be done by ensuring that discharges are, where possible, timed to occur after sea ice melting is well underway (late June) but still occurring, to allow for maximum dilution. Under-ice fauna typically abandon this habitat to become pelagic or benthic as freshwater input from melting ice increases (Pike and Welch, 1990). In addition, although juvenile amphipods tested in this study were no less tolerant to lead and zinc than adults, prudence advises avoiding sensitive reproductive periods, specifically the late fall when most breeding occurs and early spring when young are released (Riebel, 1984; Sainte-Marie et al., 1990). Thus, discharge should ideally not occur beyond late summer when sea ice begins to reform.

The above recommendations are provided as guidance for controlling discharges to ensure the integrity of the arctic marine environment. Where timing of discharge cannot be controlled, other measures such as increasing the salinity of the discharge to avoid concentration under-ice could be implemented. Implementation of such "common sense" recommendations will require on-site judgement, which is difficult to "write into" a discharge permit. However, flexibility coupled with best professional judgement is a much better way to achieve the ultimate goal of environmental protection within the context of an industrialized society than are inflexible, often numerical criteria (Chapman, 1991). But, to do this, there must be enough information to make informed decisions. To this end, there is a pressing need for augmentation of the presently depauperate arctic marine toxicity data base.

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INTERACTIONS BETWEEN ALGAE AND METALS.

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Ionic and non-ionic constituents of aqueous media greatly affect the speciation, and hence the toxicity, of heavy metal ions. Although "free" heavy metal ions are often considered the most toxic forms, heavy metals can often exert toxic effects on microorganisms when no free heavy metal ions can be detected, due to their complexation with ingredients in the culture medium or with those produced by the microorganisms themselves.

Changes in speciation involved in heavy metal (mainly copper and cadmium) interactions will be considered in discussing some of our past and current work on toxic effects on growth and division patterns of algae and cyanobacteria (blue-green algae). The role of extracellular and intracellular binding in copper tolerance of the cyanobacterium, Anabaena 7120 will also be described.

QUALITATIVE AND QUANTITATIVE RELATIONSHIPS OF MICROTOX DATA WITH ACUTE AND SUBCHRONIC TOXICITY DATA FOR OTHER AQUATIC SPECIES.

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ABSTRACT

Qualitative and quantitative relationships of Microtox data with acute and subchronic toxicity data for other aquatic species. **Can. Tech. Rep. Fish. Aquat. Sci.**

Regression analyses of the inhibitory effects of individual organic chemicals on *Photobacterium phosphoreum* (the Microtox™ test) with their acute toxicities to a variety of aquatic species including cold, warm and tropical water fish species show high degrees of collinearity over several orders of magnitude. The highest number of data pairs are found between the Microtox test and the 96-hr acute lethality test to fathead minnow (*Pimephales promelas*), a North American warm water fish which has extensively been used for testing chemicals. For this fish, the regression analysis indicates a highly significant degree of correlation over a toxicity range of nearly ten orders of magnitude. A group of regression outliers is identified by a common feature of their chemical structures.

Other species for which acute toxicities are quantitatively compared with the luminescent bacteria test are the freshwater fish goldorfe (*Leuciscus idus melanotus*), the seawater sheepshead minnow (*Cyprinodon variegatus*), and the water flea (*Daphnia magna*). Subchronic toxicity data (32-day LC50) for the fathead minnow are also investigated vis-a-vis the Microtox test. The regressions for all species including fathead minnow vary little in slope with a slope of 0.68 for sheepshead minnow being the smallest and a slope of 0.90 for goldorfe being the largest. The corresponding intercepts vary from -0.20 to 0.29 for the acute toxicity regressions as compared to an intercept of 1.32 for the regression of subchronic fathead minnow data versus Microtox.

Together with the recently published *Photobacterium phosphoreum* toxicity data index, these results provide for the quick estimation of the effects of a large number of chemicals on the above and other aquatic species. In the absence of detailed experimental data, such estimates are of considerable value for regulatory initiatives, monitoring and environmental spill control actions.

KEY WORDS: Acute toxicity, subchronic toxicity, *Photobacterium phosphoreum, Pimephales promelas, Leuciscus idus melanotus, Cyprinodon variegatus, Daphnia magna*, Microtox, inter-species toxicity relationships.

INTRODUCTION

The desire to substitute the use of mammals and fish for *in vivo* bioassays with lower organisms as well as the need for faster and more inexpensive test systems, has led to the introduction of standardized bacterial test systems, such as with *Photobacterium phosphoreum*, commonly known as the Microtox™[1] test. With the recent publication of a substantial toxicity data set for this test, covering well over one thousand organic chemicals (Kaiser and Palabrica, 1991), the need for and the interest in inter-species comparisons of various bioassays with the Microtox test has become more urgent. In earlier investigations, such as by Curtis et al., (1982), De Zwart and Slooff (1983), such inter-species comparisons generally suffered from a lack of breadth of chemical classes, a narrow toxicity range, or from statistical limitations. Therefore, it was difficult or impossible to draw any generalized conclusions with confidence.

Recently, Kaiser and Esterby (1991) have reported on regression and cluster analyses of Microtox data for well over 200 organic chemicals with the acute lethal bioassay data for fathead minnow (*Pimephales promelas*), several other aquatic species and acute lethal oral dose data for the rat. Their results showed high collinearity between the aquatic species bioassays and the Microtox data over a range of approximately eight orders of magnitude. The present report builds upon these observations on an expanded data base (a total of 395 organic chemicals) and investigates relationships with acute and subchronic tests which have widely been used to assess the toxicity of chemicals in temperate climates.

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[1] Microtox is a registered trademark of Microbics Corporation, Carlsbad, CA, USA.

EXPERIMENTAL AND DATA

Microtox Data

The Microtox data (in order of preference for 30-min, 15-min, or 5-min exposure) were taken from the recent index by Kaiser and Palabrica (1991). A very few additional data, not published in that compilation were determined in this laboratory under the same general conditions as for the above.

Aquatic Bioassay Data

All aquatic bioassay data are LC_{50} values taken from the literature as given in Table 1. Where possible, data were taken from reports covering larger data sets produced under standard conditions to avoid inter-laboratory variations due to differences in water hardness, temperature, species acclimatization and so forth. Details as to the test species, test conditions and sources are also summarized in Table 1.

Table 1. Aquatic species, test conditions (s=static, f=flowthrough), climatic preference and data sources used.

Species	Latin Name	Conditions & Environment	Source
Microtox™	*Photobacterium phosphoreum*	30-min, EC_{50}, s, 15°C	Kaiser & Palabrica, 1991
Fathead minnow	*Pimephales promelas*	96-hr LC_{50}, f, warm	Geiger et al., 1985, 1986, 1988, 1990,
Fathead minnow	*Pimephales promelas*	32-d LC_{50}, f, warm	Call & Geiger, 1992
Goldorfe	*Leuciscus idus melanotus*	48-hr LC_{50}, s, warm	ISHOW database 1983
Sheepshead minnow	*Cyprinodon variegatus*	96-hr LC_{50}, s, warm	Heitmuller et al., 1981
Water flea	*Daphnia magna*	48-hr LC_{50}, s	Bazin et al., 1987; Deneer, 1988; Devillers, 1988; LeBlanc, 1980; Thurston et al., 1985; Van Leeuwen et al., 1985; Steinhäuser et al., 1985; Vighi and Calamari, 1985

Data Transformations

All toxicity data have been transformed from the commonly given mg/L form into the negative logarithms of the millimolar concentrations. This presentation provides both a common (molarity based) notation and has the advantage of showing more toxic chemicals with higher values.

RESULTS AND DISCUSSION

Statistical Overview

As shown in Table 2, the largest number (count) of fish acute toxicity data for individual chemicals in common with inhibition data for *Photobacterium phosphoreum* is found for the fathead minnow (n=248). This species has been used for a number of years to systematically test hundreds of chemicals (Geiger et al., 1986, 1988, 1989, 1990). Substantial numbers of data are also available for the *Daphnia magna* (n=186), the goldorfe (n=85), the sheepshead minnow (n=32), and the subchronic fathead minnow (n=20).

It should be noted here that all data were taken from the literature without any preselection. Therefore, at least from the point of the analyses presented here, there is no bias as to the types or classes of chemicals represented in each set. Studies on the chemical classification of the investigated compounds will be undertaken in the future.

Table 2 also gives the minimum, maximum and range of observations for each species. The toxicity ranges observed for each species is between 3.1 and 8.4 orders of magnitude, expressed in molar concentration terms. For the fathead minnow, the largest data set, this range is 8.3 orders and the range for the corresponding Microtox values 10.0 orders of magnitude. Histograms (not shown) of the fathead minnow and Microtox data indicate close to normal distributions in each of the data sets. Hence, the results of regression analysis represent significant relationships which are not unduly influenced by a few influential observations. This test for robustness of the data set(s) has repeatedly been overlooked in the literature. Also for the other species fairly normal data distributions are evident.

Table 2. Statistical and regression results before outlier rejection.

	Count	Minimum	Maximum	Range	Slope	Intercept	r	r^2	Standard Error
Independent variable:									
Microtox	395	-4.00	5.96	9.96	n/a	n/a	n/a	n/a	n/a
Dependent variable:									
Fathead minnow (acute)	248	-2.95	5.34	8.29	0.79	0.04	0.775	0.60	0.92
Daphnia magna	186	-2.87	5.76	8.63	0.86	0.11	0.854	0.73	0.85
Goldorfe	85	-2.25	6.16	8.41	0.86	-0.23	0.877	0.77	0.81
Sheepshead minnow	32	-0.62	2.43	3.05	0.59	0.30	0.663	0.44	0.64
Fathead minnow (subchronic)	20	-0.85	6.36	7.21	0.76	1.32	0.854	0.73	0.93

Regression Analyses

Table 3 gives the results of linear regression analyses for each fish species versus the corresponding Microtox data. Given are the slope, intercept, correlation coefficient (both "r" and "r^2") and standard error of the estimate for each regression using all compounds available for each case. All regressions for the data are highly significant at significance levels of 99.9% or higher.

Table 3. Statistical and regression results after outlier rejection

Dependent variable	Count	Outliers removed	Slope	Intercept	r	r^2	Standard Error
Fathead minnow (acute)	235	13	0.81	-0.08	0.849	0.72	0.73
Daphnia magna	175	11	0.85	0.08	0.849	0.72	0.63
Goldorfe	82	3	0.90	-0.20	0.894	0.80	0.63
Sheepshead minnow	29	4	0.68	0.29	0.860	0.74	0.40

Figure 1 gives a plot of the fathead minnow versus Microtox data for all 235 data pairs. The 95% confidence interval for the corresponding regression (Table 3) indicated 13 outliers, which are shown as solid squares in the figure. After removal of these outliers, the goodness of fit improved, as measured by the regression coefficient (standard deviation), from r= 0.775 (s= 0.92) to r= 0.849 (s= 0.73). An investigation of the indicated outliers is given further down. Similar plots of the regressions after outlier rejection are given in Figure 2 for the *Daphnia magna* (11 outliers), Figure 3 for the goldorfe (three outliers), and Figure 4 for the sheepshead minnow (four outliers).

As apparent from even a cursory visual comparison of Figures 1 to 4, the data for all compounds and species are predominantly found near the 1:1 normal through such plots. The corresponding regressions after outlier rejection are summarized in Table 3.

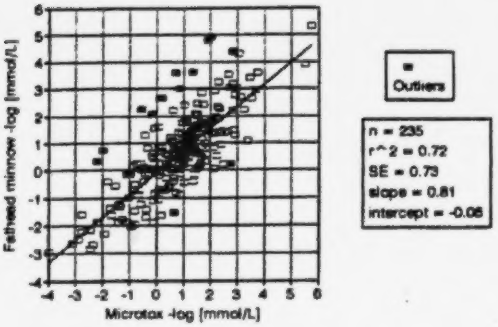

Figure 1. Plot of the acute toxicities of 248 organic chemicals to the fathead minnow versus the Microtox test and regression data.

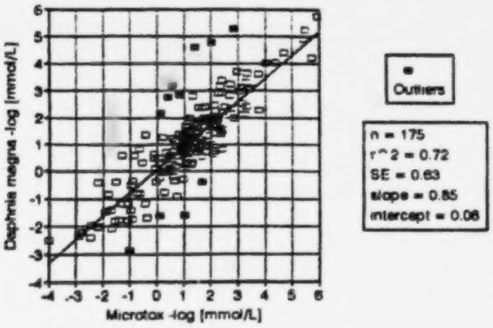

Figure 2. Plot of the acute toxicities of 186 organic chemicals to *Daphnia magna* versus the Microtox test and regression data.

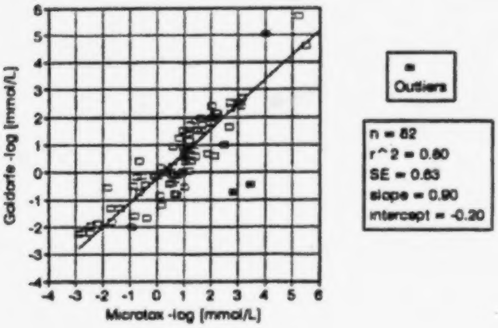

Figure 3. Plot of the acute toxicities of 85 organic chemicals to the goldorfe versus the Microtox test and regression data.

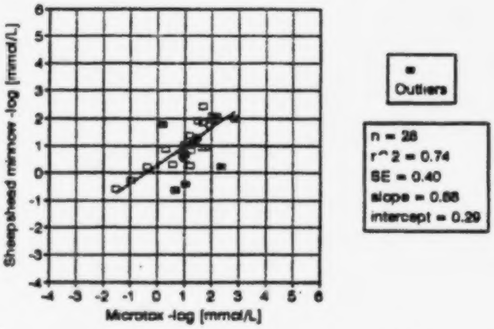

Figure 4. Plot of the acute toxicities of 32 organic chemicals to the sheepshead minnow versus the Microtox test and regression data.

Subchronic Toxicity

On examination of Figure 5, it becomes apparent that the Microtox test can also have a large degree of success in predicting the subchronic toxicity of aquatic fish species as well as their acute counterparts. In the case of the fathead minnow, the slope remains virtually unchanged (see Figure 6), at 0.81 for the acute fathead minnow and 0.76 for the subchronic fathead minnow data. The intercept for the subchronic fathead minnow is 1.32 whereas it is -0.08 for the acute fathead minnow. Since the chronic assay is for 32 days as compared to an acute assay over 96 hours, this larger intercept is to be expected.

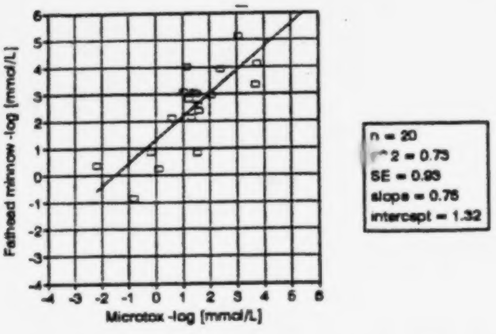

Figure 5. Plot of the 32-day LC_{50} subchronic toxicities of 20 organic chemicals to the fathead minnow versus the Microtox data and regression data.

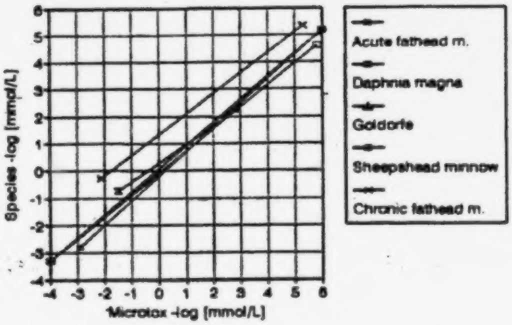

Figure 6. Plot of the regression slopes of acute toxicities to the fathead minnow, sheepshead minnow, goldorfe and *Daphnia magna* (Figures 1 to 4), and the subchronic toxicities to the fathead minnow (Figure 5) versus the Microtox test.

Correlations Between Species

A more accurate comparison of the various regressions can be made by plotting the individual regression lines over the experimental data ranges as shown in Figure 6 showing the regressions as given in Table 3 in a generalized graph of toxicity to fish and *Daphnia magna* versus Microtox.

As evident from Figure 6, there is an extremely close overlap between the fish and *D. magna* tests in both slopes and intercepts. This indicates the extremely good correlations between the various toxicity tests and the Microtox test. These results demonstrate a very high degree of commonality between the Microtox and the acute toxicity tests as well as a slope commonality for the subchronic fathead minnow data.

Chemical Features of Outliers

As indicated in Table 3 and Figures 1 to 4, the regression analyses indicated several outliers. The highest number of outliers (13) is found for the fathead minnow correlation, which represents approximately 5% of the fathead minnow data set. Examination of the chemical features of the outliers reveals that several, but not all, have terminal =C$_2$ groups, where R= H or Cl.

A search of all chemicals common to the fathead minnow and Microtox data sets shows that there are thirteen compounds containing this terminal =CR$_2$ group. All of these chemicals exhibit a higher toxicity towards fathead minnow than expected from the regression model with *Photobacterium phosphoreum*. Figure 7 shows quantitatively the deviation of the calculated from the measured toxicities.

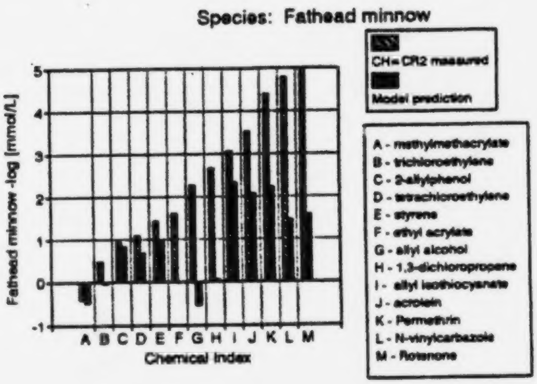

Figure 7. Bar graph of measured acute toxicities of chemicals with the =CR$_2$ (R= H, Cl) group to fathead minnow versus those predicted from the regression shown in Figure 1.

CONCLUSIONS

In summary, this study demonstrates extremely high linear correlations of the Microtox test with both acute and subchronic toxicities of a large variety of organic chemicals. to several species, including freshwater and seawater species.

Compounds with certain functional groups, such as the terminal $=CH_2$ group, can exhibit higher toxicity to fish than expected from the corresponding Microtox values.

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ENVIRONMENT CANADA'S TOXICOLOGICAL TEST METHOD DEVELOPMENT PROGRAM. R. Scroggins, Environment Canada, Hull, PQ; and G. Sergy, Environment Canada, Edmonton, AB.

Commencing in 1988, a program was initiated to develop standardized toxicological test methods appropriate for Environment Canada's aquatic environmental monitoring and regulatory needs. Standard tests for determining the acute lethality of chemicals, pesticides, effluents, elutriates, leachates and ambient waters to rainbow trout, threespine stickleback, and *Daphnia magna* have been published under the guidance of Canada's federal/provincial Inter-Governmental Aquatic Toxicity Group (IGATG). In early 1992, a multi-use test method for reproduction inhibition and survival using *Ceriodaphnia dubia* and a test for fathead minnow larval growth inhibition and survival was also officially released. Environment Canada toxicological test methods slated for release during 1992 include: a rapid toxicity test using the luminescent bacterium; a microplate test for growth inhibition using the green alga, *Selenastrum capricornutum*; a sediment lethality test using marine or estuarine infaunal amphipods; a test for fertilization success using echinoids and an early life stage toxicity test for salmonids. During 1993, the current plan is to focus method development efforts in the areas of freshwater sediment and macro-plant toxicity tests. The presentation will describe the method development process used successfully to-date and efforts to remain compatible with relevant international methods and standard guides.

ENVIRONMENT CANADA'S NEW FERTILIZATION ASSAY WITH GAMETES OF SEA URCHINS OR SAND DOLLARS.

J.B. Sprague, Sprague Assoc. Ltd., Guelph, ON, (519) 763-0263, D.J. McLeay, McLeay Assoc. Ltd., West Vancouver, BC, R.P. Scroggins, Environment Canada, Ottawa, ON, and G.A. Sergy, Environment Canada, Edmonton, AB.

EXTENDED SUMMARY

Environment Canada will soon publish a method for toxicity tests based on fertilization in echinoids (Environment Canada, in press). The test is recommended for general use and is specified in the new pulp and paper effluent regulations. The document will be "generic", but gives preferred choices among alternatives for species, exposure times and test volumes; any Canadian regulatory test would select a particular method from the generic alternatives.

The Environment Canada methods were chosen on the basis of literature review, guidance of the Inter-Governmental Aquatic Toxicity Group (IGATG), comments of a large group of reviewers, and experience in a Canadian inter-laboratory trial (Miller et al., in press). Methods have previously been described by the BC Ministry of the Environment, Beak Consultants Ltd. and E.V.S. Consultants. The Environment Canada method attempted to correspond with these and with methods developed by the USA Environmental Protection Agency, American Society for Testing and Materials, and National Council of the Paper Industry for Air and Stream Improvement, Inc.

The test has many useful features. It is suitable for chemicals, surface waters, and wastewaters (effluent, leachate, elutriate, or interstitial water from sediments). It uses ordinary facilities. Success of fertilization is a susceptible and important part of the life cycle. This is among the most sensitive of marine sublethal tests. It is rapid (20 min) and small in volume (10 mL), so it is useful for pilot-scale studies or toxicity identification, and only small volumes of effluent need be shipped to a distant laboratory. Echinoids are common, frequently studied and biologically well-documented, easily collected and shipped, and readily held in the laboratory. The toxicity test requires seawater salinity, but freshwater samples can be tested by adding salts or brine.

Suitable native species include the green sea urchin *(Strongylocentrotus droebachiensis)* found on Atlantic, Pacific and Arctic coasts, the Pacific purple sea urchin *(S. purpuratus)*, and the eccentric sand dollar *(Dendraster excentricus)* of the Pacific. Other local echinoids may be used if they perform suitably. Exotic species may be imported under permit, such as the Atlantic sea urchin "Arbacia" *(Arbacia punctulata)* and the white sea urchin from California *(Lytechinus pictus)*. These and other Echinodermata are structurally sophisticated invertebrates, with many embryonic and biochemical similarities to chordates.

Adult echinoids may be held in the laboratory for months in ordinary tanks. Sea urchins are fed kelp, macroalga or romaine lettuce. Sand dollars normally ingest particles from the sand or sediment on the bottom of their tank and feed on the organic detritus, particularly microalgae. For much of the year, gametes for testing may be obtained from

native species by choosing holding temperatures that encourage early or late spawning. Imported echinoids can allow tests in the off-seasons. In general, the various species are similar in sensitivities to toxicants; Canadian comparisons are discussed in the following paper by Miller et al. (this workshop).

The echinoid fertilization assay is sensitive. It should generally predict the results of a chronic exposure because the gametes are usually the most sensitive of the developmental stages, and are often the most vulnerable stage of the life cycle. Sensitivity is comparable to other marine and freshwater sublethal tests, such as embryo-larval tests with oyster, crab, squid, and fish. The assay is quite sensitive to metals, but less so to pesticides. For pulp mill wastes, echinoid tests were up to 10-fold more sensitive than growth and development tests with larval shrimps and fish, equal to reproduction of red alga, and an order of magnitude less sensitive than embryo-larval tests with oysters. This sublethal test should therefore be considered powerful and meaningful. Precision seems comparable to other sublethal tests and chemical measurements; Canadian findings on precision and inter-laboratory variation are discussed by Miller et al. (this workshop).

Spawning is induced by injecting KCl into the body cavity, and the collected gametes are useful for 2 to 4 hours. Semen or eggs are collected from several echinoids and pooled before use.

Tests may be run with a control and five concentrations of test substance to determine thresholds of effect, or with one concentration as regulatory or pass/fail tests. At least three replicates are required. Test vessels are borosilicate glass vials or tubes. Test volume is normally 10 mL, with options of 5.0 mL and 2.0 mL. For valid assessment of the toxic quality, *per se*, of a given substance, salinity must be in the range 28 to 34 g/kg, pH 7.5 to 8.5, dissolved oxygen above 40% of saturation, and temperature 15°C for the native species or 20°C for listed non-native species. Wastewaters could be tested without adjustment, however, with salinity, pH and oxygen values outside those limits, if it were desired to assess the total effect of a discharge.

A standard test exposes sperm for 10 min, adds eggs, and continues for 10 min more to allow fertilization, i.e. a 20-min test. Exposures totalling 40 or 80 min may also be used. The test is terminated by adding preservative to the vials. Numbers of fertilized eggs are counted in samples, as judged by raised "fertilization membranes". The control must yield ≥50% and <100% fertilization for a valid test. Less-than-complete fertilization is required in the control since an excess of sperm can mask toxicity and reduce sensitivity of the test. About 2,000 eggs are used per 10-mL vial, and the optimum sperm-to-egg ratio is determined by trial in each laboratory, as that which gives 90% fertilization under control conditions. The ratio of sperm to eggs is often in the range 50:1 to 2,500:1, but sometimes as high as 20,000:1.

Percent fertilization is calculated for each test vessel, and used to assess adverse effects on fertilization compared to the controls. The result is expressed as the *inhibiting concentration for a specified percent effect (ICp)*, or the *no-observed-effect* and *lowest-observed-effect concentrations* derived by hypothesis-testing. In a single-concentration test, an adverse effect would be lower fertilization than in controls, as determined by t-test. A reference toxicant must be tested at least once a month, and results must fall within specified limits (±2 SD), if other tests are to be considered valid. Copper is the recommended reference toxicant.

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INTER-LABORATORY EVALUATION OF ENVIRONMENT CANADA'S NEW FERTILIZATION ASSAY WITH ECHINOIDS (SEA URCHINS AND SAND DOLLARS). J.A. Miller, E. Jonczyk, D. Hart, and G.R. Craig, Beak Environmental Consultants Ltd., 14 Abacus Rd., Brampton, ON, (416) 794-2325.

Environment Canada has recently developed a biological test method entitled "Fertilization Assay with Echinoids (Sea Urchins and Sand Dollars)" to be used for the assessment and control of potentially toxic substances in a marine or estuarine environment.

A series of inter-laboratory trials with six species of echinoids (four sea urchins and two sand dollars) were conducted to investigate the relative sensitivity of different test species and the options permitted in Environment Canada's method. The effect of two sperm to egg ratios, three control fertilizations and three test durations on the sensitivity and precision of the fertilization assay was examined using a reference toxicant (copper sulphate). The sensitivity and precision of the assay were also investigated using samples of biotreated bleached kraft mill effluent and copper sulphate. The variation in biological response attributable to the test method itself as opposed to that resulting from general inter-laboratory differences (i.e., water quality) was also explored.

A RAPID, INEXPENSIVE METHOD FOR EVALUATING TOXICITY OF SOIL USING THE NEMATODE AND MICROTOX TESTS. Martin R. Samoiloff and Clifton E. Samoiloff, BioQuest International, Winnipeg, MN.

Standard artificial soil was spiked with one of 20 known organic or inorganic toxic chemicals. The soils were extracted with water (2 parts water by weight: 1 part soil by weight: and with methanol (2 parts water by weight: 1 part soil by weight) for 24 hours, then tested for toxicity using the Microtox and the nematode tests. Water extracts were tested at 10% for both tests. Methanol extracts were tested at 3% with the nematode test and at 1% using the Microtox test. In general, organic compounds showed toxicity only in the water extracts, while inorganic compounds only showed effects in aqueous extracts.

This approach provides a cost-effective, sensitive method for evaluating toxicity of soil samples and reduces the potential for false negatives associated with performing only chemical analyses.

APPLICATION OF ENVIRONMENT CANADA'S GROWTH INHIBITION TEST USING THE FRESHWATER ALGA *SELENASTRUM CAPRICORNUTUM*. J.S. Hansen and J.S. Goudey, HydroQual Laboratories Ltd., #1, 6125 - 12th St., SE, Calgary, AB, T2H 2K1, (403) 253-7121.

The algal growth inhibition test is one of a battery of standardized protocols selected by Environment Canada. Exponentially growing populations of *Selenastrum capricornutum* are exposed to the test solution in 96 well microplates for 72 h under continuous light. We report here our experiences applying this test to assess the phytotoxicity of pulp mill and mining effluents, municipal wastewater, groundwater and soil elutriates. Information on the growth of *Selenastrum* in microplates and conditions for optimal growth are reviewed. Analysis of data based on measurements of optical density and cell counts are compared and discussed in the determination of endpoints (biostimulation and growth inhibition). We have also adapted this protocol to measure biologically available nitrogen and phosphorus in aqueous samples (biostimulation).

CILIATED PROTOZOA AS TEST ORGANISMS FOR TOXICITY BIOASSAYS. G.L. Gilron, Sentar Consultants Ltd., Suite 100, 6846 King George Hwy., Surrey, BC, V3W 4Z9, (604) 597-0332; and D.H. Lynn, Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1.

Ciliated protozoa are important as food for higher tropic levels and as recyclers and remineralizers of organic material in the benthic and pelagic realms of aquatic ecosystems. Furthermore, they have been well studied, are relatively easy to culture in the laboratory, and have very rapid growth rates. For these reasons, and given the proliferation of new bioassay technologies for organisms "lower" in aquatic food chains, ciliates have become useful subjects in toxicological research, and more recently, as test organisms for bioassays. Ciliate bioassays have been developed by a number of researchers worldwide, over the past 20 years. These bioassays have utilized a variety of test organisms, and, as is the case with other toxicological tests, a variety of lethal and sublethal indicator parameters. Among these are morphology, mortality, growth, motility, respiration, genotoxicity, and chemotaxis. This paper summarizes and reviews the most recent bioassays using ciliates, in terms of their practicability and feasibility.

VARIABILITY IN BIOASSAY ENDPOINTS USING FOUR SPECIES OF INVERTEBRATES AND SEDIMENT COLLECTED FROM FIFTY UNCONTAMINATED REFERENCE SITES IN THE LAURENTIAN GREAT LAKES. K.E. Day, Rivers Research Branch, N.W.R.I., Environment Canada, Burlington, ON; T.B. Reynoldson, Lakes Research Branch, N.W.R.I., Environment Canada, Burlington, ON; R. Norris, University of Camberra, Australia; R. Bailey, Western University, London, ON.

A three-year study is currently underway at the National Water Research Institute to develop biological sediment guidelines for the nearshore areas of the Laurentian Great Lakes. This study has several program objectives amongst which is the development of a classification system for unpolluted habitats based on species composition of the benthic community and the responses from four sediment toxicity tests using infaunal and epibenthic invertebrates. In the first year of the study, sediments collected from fifty uncontaminated nearshore (<30 m) areas in Lakes Erie, Ontario, Huron and Superior have been used in bioassays to measure survival and growth of *Chironomus riparius*, *Hyalella azteca* and *Hexagenia* spp. as well as reproduction of *Tubifex*. Percent survival for the three species ranged from 60.0-95.6 for *C. riparius*, 86.7-100.0 for *H. azteca* and 83.3-100.0 for *Hexagenia*. Growth ranged from 0.16-0.55 (dry weight), 0.24-20.9 (wet weight) and 0.39-0.68 (dry weight) for each of the three species. Total production of offspring for *T. tubifex* was 63.8-176.3 over 28-d. The implications of these ranges in responses with regard to the detection of toxicity for contaminated sediments will be discussed.

ECOLOGICAL EFFECTS TESTING UNDER THE TOXIC SUBSTANCES CONTROL ACT: ACRYLAMIDE*. John D. Walker, Office of Toxic Substances (TS-792), USA Environmental Protection Agency, 401 M St., SW, Washington, D.C. 20460.

Analysis of acrylamide aquatic toxicity data submitted under section 4 of the Toxic Substances Control Act revealed that for acute toxicity studies with bluegill, fathead minnow, and rainbow trout, concentrations of acrylamide necessary to produce 50% mortality decreased by 50-70% as a function of increasing exposure from 1 to 4 days. Analysis of acute toxicity data for *Mysidopsis bahia* suggested that increases in mortality with increased exposure to acrylamide might also be observed in saltwater organisms. Ratios of acute (4-day) LC_{50} values to chronic (28-day) maximum acceptable toxic concentrations for this saltwater invertebrate were 26 for parent and offspring survival, 115 for female dry weights, and 975 for male dry weights. These ratios illustrate that long-term acrylamide exposure to sensitive life stages of *M. bahia* produced adverse effects on reproduction and growth at acrylamide concentrations significantly lower than those suggested by acute LC_{50} or EC_{50} values.

IN-SITU MULTI-SPECIES APPROACH TO ENVIRONMENTAL EFFECTS MONITORING OF A MAJOR RIVER ECOSYSTEM.

T. Moran, M. Lines, K. Getty, Pollutech Environmental Limited, Sarnia, ON, (519) 339-8787, T. Kierstead, Lambton Industrial Society on behalf of Novacor Chemicals (Canada) Limited, Sarnia, ON.

Abstract

The St. Clair River is both a major route for Great Lakes shipping and an international boundary between the province of Ontario and the state of Michigan. Along the Ontario side of the river is a series of petrochemical, organic and inorganic industrial complexes. These plants along with urban and agricultural sources discharge to the St. Clair River. To determine the environmental impact of these discharges a multi-species bioassay study was conducted. In-situ monitoring included the use of Rainbow Trout (*Oncorhynchus mykiss*) eggs for determining hatchability success from the green to swim-up stage; *Daphnia magna* and *Daphnia pulex* life-cycle testing for determining survival and reproductive rates; and, Fathead Minnows (*Phimphales promelas*) for determining fecundity rates. The experimental design included a control station located upstream of all industrial and urban inputs and a comparable downstream station. The series of toxicity tests were conducted using a flow-through apparatus that allowed for monitoring of the river water quality including routine discharges and spill events. Methods, apparatus and results for the Rainbow Trout egg tests from 1989 to 1991 including preliminary results for 1992 will be discussed.

INTRODUCTION

Along the Ontario side of the St. Clair River are located 15 major industrial facilities which include petroleum refineries, petrochemical, organic, and inorganic plants and thermal electric generating facilities all of which discharge directly or indirectly to the St. Clair River. Other sources of discharges include several municipal wastewater treatment facilities, urban and agricultural non-point sources, and combined sewer overflows. Total flow from point source facilities represents approximately 20 percent of the river's daily flow. Of this, 97 percent is industrial once-through or non-contact cooling water (OMOE and MDNR, 1991).

Extensive environmental monitoring of the river has been ongoing since the mid 1950's. Programs have included examination of the sediment, fish, benthos, water chemistry, flow dynamics, surface contamination, and the river as a drinking water source, etc. (LIS, 1989). Historical studies have indicated a degraded environment characterized by poor water quality and sediment contamination. More recent studies and examination of overall trends indicate that the St. Clair River environment is continually improving (OMOE and MDNR, 1991; LIS, 1989). These improvements in the St. Clair River environment have been attributed to reduced contaminant loading to the river and the consequent rebound effects (OMOE and MDNR, 1991).

The Lambton Industrial Society (LIS), an environmental cooperative of the major Sarnia area industries, has been instrumental in demonstrating the improvement of the St. Clair River environment by sponsoring programs of environmental monitoring and urging improvement. As part of its commitment to the St. Clair River environment, the Lambton Industrial Society has commissioned a number of studies investigating water quality. Some of these studies have included fish taste and odour testing, benthic macroinvertebrate studies, Rainbow Trout growth studies, fish flesh analysis, histopathological investigations, caged fish studies, automated, remote on-line chemical analysis and sediment toxicity assessments. The LIS has supported innovative work that would complement concurrent studies and address public concerns.

The approach of the LIS biomonitoring program is unique in many ways. The multi-species approach recognizes that different species can react very differently to the same level of contamination. The multi-species approach provides a "burden of evidence" as to the impact contaminants have on the biological water quality of the river.

The LIS biological monitoring program incorporates a flow-through design. This design evaluates the biotic response to water conditions which exist during the entire monitoring period, on a continuous basis.

Biomonitoring stations were located at two fixed sites, located on the banks of the St. Clair River (Figure 1), to which river water was continually supplied. The upstream site, located just south of the mouth of the river received water entering the river from Lake Huron. Incoming water from the upstream location represented the background water quality before receiving any discharge waters. The downstream site, approximately 25 km south of Lake Huron, received water that contained the contributions of all point source and non-point source discharges from the Sarnia-Lambton urban, rural, and industrial areas. Data from the two biomonitoring stations were compared to determine the net effect of river water quality on all studied species.

As the quality of the St. Clair River has improved biomonitoring studies have focused on multi-species assessments of water quality that monitor sublethal and chronic endpoints. The test organisms chosen were *Daphnia magna*, *Daphnia pulex*, Fathead Minnows (*Pimephales promelas*) and Rainbow Trout (*Oncorhynchus mykiss*). These well studied organisms represented various levels of the trophic system within the mainstream aquatic environment.

This paper summarizes the endpoints and results for all of the studies conducted. Details of experimental design, methods, and results of the Rainbow Trout egg hatchability experiments conducted annually from 1989 to 1991 are documented. Preliminary results for 1992 Rainbow Trout egg evaluations are also presented.

METHODS

Program Scheduling

The Rainbow Trout study was conducted during the spring of each study year, when water temperature was below 20°C. The duration of the Rainbow Trout egg exposure varied from 30-36 days due to yearly fluctuations in water temperature. The *Daphnia* studies were conducted for two 21 day exposures when the temperature of the river approached 20°C. The Fathead Minnow studies were conducted from May to October when egg production was likely to occur.

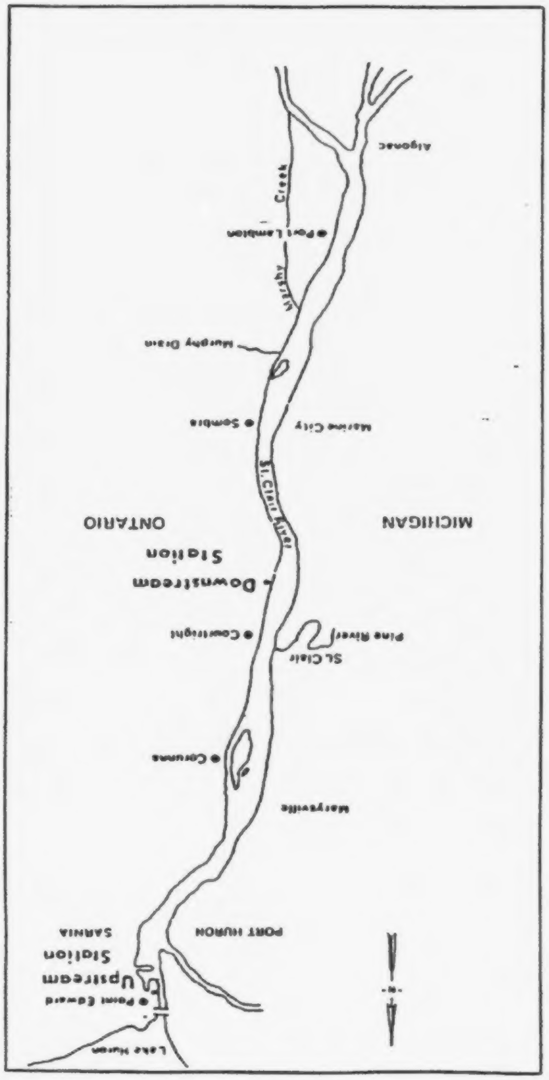
Algonac
Creek
Port Lambton
Marshy
Murphy Drain
Sombra
Marine City
St. Clair River
ONTARIO
MICHIGAN
Downstream Station
Courtright
Pine River
St. Clair
Corunna
Marysville
PORT HURON
SARNIA
Upstream Station
Point Edward
Lake Huron
N

Figure 1. Biomonitoring Station Locations

Daphnia magna, *Daphnia pulex* Survival and Reproductive Study

The *Daphnia* species were chosen as representatives of the zooplankton community. The selection of daphnids for use in bioassays is appropriate for many reasons including being: indigenous to freshwater bodies throughout Canada; an important link in many aquatic food chains; and, a significant source of food for juvenile stages of salmonoid and other fish species. Daphnids also have a relatively short life cycle, are easy to culture, are documented as being sensitive to a broad range of aquatic contaminants and are widely used as test organisms for evaluating toxicity of chemicals, effluents, and surface waters (Environment Canada, 1990a). The *Daphnia* studies were conducted to determine water quality effects on reproductive success and survival.

Fathead Minnow Life-Cycle Effects Monitoring

The Fathead Minnow is also a prominent test species in the field of aquatic toxicology. It has been used for life-cycle tests in the United States since the 1960's (Mount and Stephen, 1967) and is now a standard species for tests of both acute lethality and sublethal effects (Weber et al., 1989; Environment Canada, 1990b). Key life stages of embryo, larva, and adult are recommended for bioassays of sediment, effluents, elutriates, leachates, chemicals and surface waters (Environment Canada, 1990b). The Ontario Ministry of the Environment has recently proposed the requirement for Fathead Minnow chronic toxicity testing of industrial effluents under the Municipal Industrial Strategy for Abatement program (Ontario Ministry of the Environment, 1992). A toxicological database has been and will continue to be assembled for this test species.

Three key life stages of the Fathead Minnow (egg, larva, and adult) were observed to determine whether water quality had any impact on adult survival, egg production, egg hatching and larval growth.

Rainbow Trout Egg Hatching Trials

Rainbow Trout (*Oncorhynchus mykiss*) are native to western North America. As a result of both intentional and accidental releases, this species now inhabits waters of all Canadian provinces where it thrives in most cool, fresh waterbodies. The species has been introduced around the world with considerable success and now is probably the most widespread of the salmonids. The Rainbow Trout has also become the world's standard cool-water fish for freshwater pollution studies and research in aquatic toxicology. Rainbow Trout have been used extensively in Canada for evaluating short-term toxicological effects since the 1960's.

Rainbow Trout eggs were first used in a flow-through bioassay in 1988. Results of the initial experiment demonstrated that the eggs could be incubated in a flow-through system of river water with survival close to levels expected under hatchery conditions (Pollutech, 1988). Since 1988, the Rainbow Trout egg hatching study has been included in the annual LIS biological monitoring program. From these studies a sizeable database has been assembled of background water quality information for the St. Clair River.

The objectives of the Rainbow Trout egg study were to determine whether differences existed in the response and survival of eggs between stations, if filtering the water had an effect on egg survival and if a correlation with rainfall events and egg survival existed.

Some of the advantages of the Rainbow Trout egg hatching study included:

- **the development of a biomonitoring technique for continuous assessment of water quality;**
- **monitoring stages with different sensitivities; animals in the early stages of development have been found to be more sensitive than adults (McKim, 1977; Macek and Sleight, 1977; Norberg and Mount, 1985);**
- **elimination of effects from feed on growth and development; food is a common source of variability in most controlled biomonitoring systems (Lanno, 1989); during early stages of development the Rainbow Trout larvae are entirely nourished by the egg yolk; and**
- **development of a test apparatus which can effectively control ambient conditions such as light, water flow and stocking density.**

Development of Rainbow Trout eggs may be divided into three distinctive stages including green to eyed, eyed to hatch and hatch to swim-up stages. Although other stages of development are known to occur, these three are distinct endpoints in the egg's developmental process. During these stages of development the eggs and embryos remain relatively immobile within the test apparatus and do not physically respond to changing water quality or physical conditions by "Behavioral Avoidance."

Experimental Design

Rainbow Trout eggs were obtained from a commercial, certified disease free hatchery. The eggs were incubated to the swim-up stage in upstream and downstream water. At each station two river water conditions were tested; raw water and filtered water. For each water treatment, 12 replicate incubator jars were used with 200 eggs in each incubator. Daily observations were made for egg mortality and to ensure proper functioning of the experimental system. River water temperature and weather conditions were recorded. Figure 2 provides an overview of the experimental design that was used for all four study years.

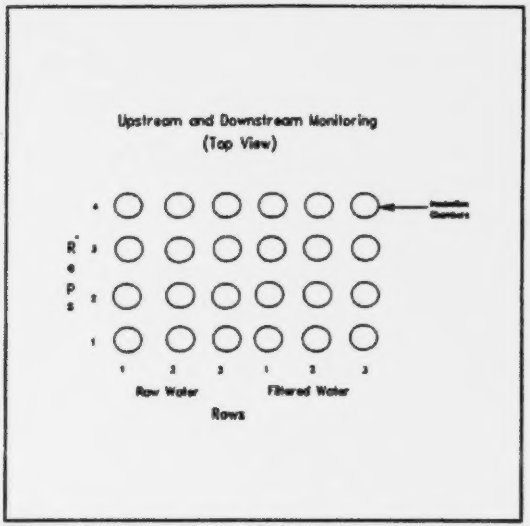

Figure 2 - Top View of the Rainbow Trout Egg Experimental Design

Test Apparatus

At each station, the eggs were exposed to either raw or filtered water. To obtain filtered water, in 1990, 1991 and 1992, river water was passed through a series of coalescing filters in the order of 80 µm, 30 µm and 5 µm. Filters were tested for adverse effects to both test daphnid species prior to the 1990 study. During the 1989 study a different method using sand filters was employed which was prone to repeated flow reductions.

In all studied years river water for the experiment was taken directly from a pumped supply available from within each station building. Water was delivered to the egg incubation apparatus via an ABS header pipe system. Individual incubator jars were fed via food grade PVC tubing fitted with clamps to regulate water flow. For 1992, changes were made to the apparatus which included the installation of gravity fed header tanks and diffusers for better flow regulation.

The incubation jars for the egg hatching experiment are shown in Figure 3. Each incubation chamber was made of two sections. The eggs were contained in a 2 L glass jar from which the bottom had been removed and replaced with stainless steel mesh. The 2 L jar was suspended by stainless steel wire inside a 4 L glass jar that had been modified by removing the top of the jar. River water was delivered into the 2 L jar from the top, flowed over the eggs, through the stainless steel mesh, into the second jar and exited over the sides of the second jar.

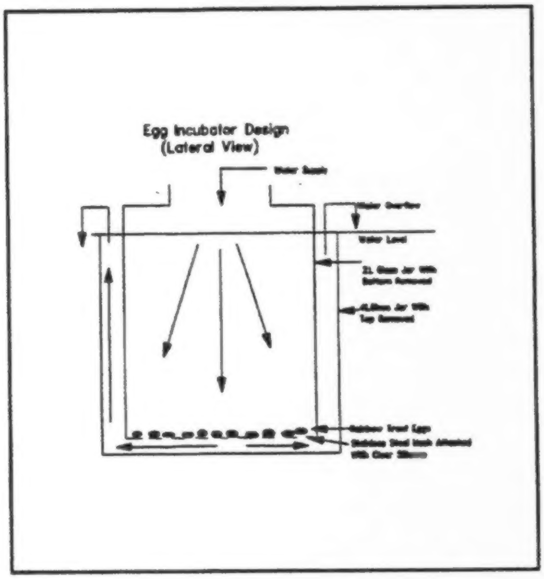
Egg Incubator Design
(Lateral View)
Water Supply
Water Overflow
Water Level
2L Glass Jar With Bottom Removed
4L Glass Jar With Top Removed
Rainbow Trout Eggs
Stainless Steel Mesh Attached With Clear Silicone

Figure 3. Lateral View of the Egg Incubation Chamber

Before re-use each season, incubation chambers were sterilized in 5% H_2SO_4, and rinsed with dechlorinated water. All materials used in the construction of the apparatus were considered inert.

During the green and eyed stages of development, the water flow to each incubator was maintained at 300 mL/min (+/- 20 mL) to minimize disturbance to the eggs. During the third stage of development, the hatched stage, water flows were adjusted to 1400 mL/min (+/- 100 mL). Higher flows are required during this stage to provide optimum oxygenation conditions to the developing larvae. The flow rates maintained were similar in all study years.

Dead eggs and larvae were counted and removed daily to prevent fungi, that developed on the dead forms, from spreading to viable ones. Dead eggs and larvae were removed using a large bore glass pipette taking care not to disturb or damage the viable ones. Periodically the incubator jars were cleaned of accumulated silt. Eggs and larvae at both stations were subjected to the same techniques.

During the incubation period, eggs and larvae, were kept in darkness. Only during daily maintenance were the eggs and larvae exposed to incandescent light. Experience showed that Rainbow Trout eggs were sensitive to light exposure over extended periods.

Statistical Analysis

Survival data were analyzed using Analysis of Variance (ANOVA). Each stage of development was analyzed at the 95% level for:

- effects between the rows and replications of the test apparatus;
- effects of filtration; and
- differences in survival upstream and downstream.

Overall survival data were analyzed for the above as well as yearly differences. Survival data for each station were also analyzed using series analysis. The time taken to reach 25% mortality (75% survival), or LT25, was a test endpoint. Water quality was measured as the time required to reach 25% mortality. More time indicated better water quality.

Immunoassays

Immunoassays were conducted for 2, 4-D, Metolachlor and Atrazine on river water samples collected during dry and wet weather conditions on May 27 and June 18, 1992 respectively. Samples were collected at the upstream and downstream stations two.

RESULTS AND DISCUSSION

Daphnia magna and *Daphnia pulex* survival and reproductive rates were studied. Fecundity rates were monitored in Fathead Minnows (*Pimephales promelas*). These evaluations did not detect any difference in the experimental endpoints between the upstream and downstream stations. Details of these studies will be available in future publications. Rainbow Trout eggs were determined to be the most sensitive biological indicator. Details of the results of these studies are discussed here.

The results of the 1989, 1990, 1991 and 1992 egg hatching experiments were calculated as percent survival for the green, eyed and hatched stage of development. At the beginning of each stage the number of eggs surviving the previous stage was taken as 100 percent. The absolute numbers of eggs were used for statistical analysis. The green and eyed stages were the most sensitive and showed the highest mortalities in comparison to the hatched stage.

Each year the water feed line to several incubation chambers periodically became clogged, thereby reducing flow. At times this decrease in flow caused increased flow in adjacent incubation chambers. The experimental effect of a severe change in flow caused some mortalities. Depending on the developmental stage affected, the resulting mortality data were treated differently.

Experience has shown that few mortalities occurred during the hatched stage in these experiments. When a change in flow caused mortalities in the hatched stage, the dead larvae were recorded. It was assumed that without this change in flow that the hatched larvae very likely would have survived. Adjustments were made in the statistical analysis for the accidental mortalities. Most mortalities that did occur in this experiment involved the green or eyed stages. When accidental mortalities from flows occurred during the green or eyed stages, results were not included in the statistical analysis. As these were sensitive stages it was impossible to know how many eggs would have survived had the flows been consistent. Data adjustment was necessary to provide an independent statistical analysis.

Difficulties in maintaining consistent flows, although infrequent, were noted at both stations during 1989, 1990 and 1991. All data for these studied years were adjusted and treated the same statistically. Although the changes made to the flow-through apparatus in 1992 were successful in reducing flow restrictions and surges, a few such events did occur.

Frequency and types of deformities were recorded at each station. Curvature of the spine, siamese twins, and two headed and two tailed larvae occurred in all treatment groups from 1989-1991. Deformities were expressed as percent of the total number of eggs in each treatment and ranged from 0.13-1.80% over the three years. Although deformities are observed in hatcheries often the values are not recorded. Comparable hatchery data was not available.

Table I shows a summary of the upstream and downstream survival data, with all stages combined, for 1989-1991. Figures 4, 5, and 6 summarize the survival data for the upstream and downstream locations by combining the results of the raw and filtered water treatment groups for 1989, 1990 and 1991. Preliminary results for 1992 are provided in Figure 7. For presentation purposes the % survival in Figure 6 is not on the same scale as Figures 4, 5, and 7.

Table 1. Rainbow Trout Egg Hatching Trials 1989, 1990 and 1991 Summary Tables Percent Survival

ROW	REPLICATION	OVERALL PERCENT SURVIVAL SUMMARY											
		UPSTREAM						DOWNSTREAM					
		UNFILTERED			FILTERED			UNFILTERED			FILTERED		
		1989	1990	1991	1989	1990	1991	1989	1990	1991	1989	1990	1991
1	1	60.3	76.4	54.4	65.9[1]	83.9	62.1	64.1	54.5[1]	27.7	62.1	60.1	N.I.[2]
	2	66.3	71.2	N.I.[2]	71.2[1]	72.6	73.1	66.3	64.3	20.9	61.6	42.7	N.I.[2]
	3	59.7	80.3	59.2	65.3	73.5	70.0	61.2	64.7	N.I.[2]	62.2	57.8	46.6
	4	58.6	65.2	37.3	66.3	76.1	59.8	66.5	75.6	N.I.[2]	69.6[1]	71.9	34.7
2	1	65.9	73.0	26.1[1]	64.6[1]	70.5	39.8	58.7	61.9[1]	34.5	60.7	52.8	31.6
	2	67.3	72.7	48.0	69.4[1]	77.9	66.2	54.7	48.6	19.8	60.0	62.9	19.0
	3	65.8[1]	73.1	43.6	59.5[1]	79.7	44.9	59.6	64.9	32.5	64.1	72.1	30.9
	4	63.3	75.7[1]	58.1	71.2	69.0	37.8	60.6	77.5	32.2	62.8	69.3	46.7
3	1	69.4	48.2	32.8	72.8[1]	61.4	60.3	66.7	58.3	29.1	63.4	58.3	16.3
	2	74.7	74.4	55.3	70.7	85.0	65.0	57.8	51.0	23.1	63.5	56.7	27.2
	3	65.6	74.7	61.2	64.0	80.2	20.3[1]	56.9	69.8	36.1	58.8	67.6	20.9
	4	64.6	39.0[1]	54.8	57.1	80.9[1]	64.7	56.4	72.6	27.1	65.6	N.I.[2]	46.8
AVERAGE		65.1	68.6	48.1	66.4	75.8	55.6	60.8	63.5	28.3	62.9	61.2	32.1

[1] Partial or total reductions in flow caused increased mortality during the hatched fry stage, embryos assumed alive until completion of the experiment; percents adjusted accordingly.

[2] Uncontrolled strong flow or loss of flow during either the green or eye stage of development caused increased mortality. The results from these incubator jars were not included.

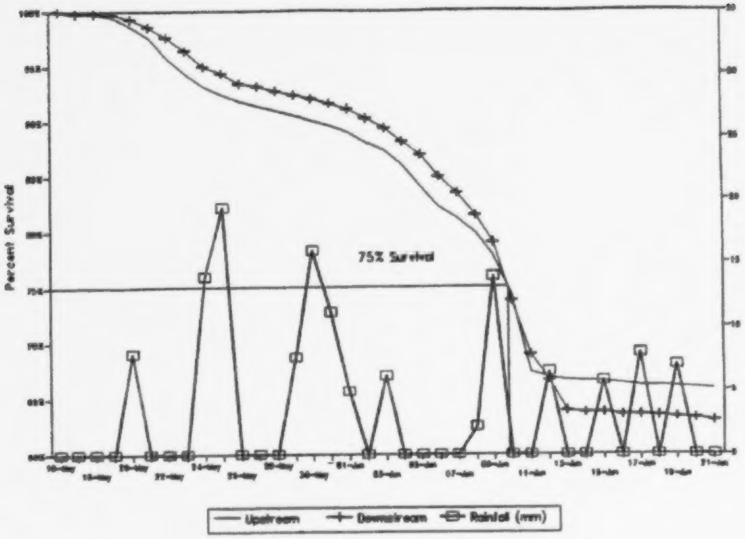

Figure 4. Overall 1989 Rainbow Trout Egg Upstream/Downstream Survival Results with Plotted Rainfall and LT25 (Time to 75% Survival) Extrapolations.

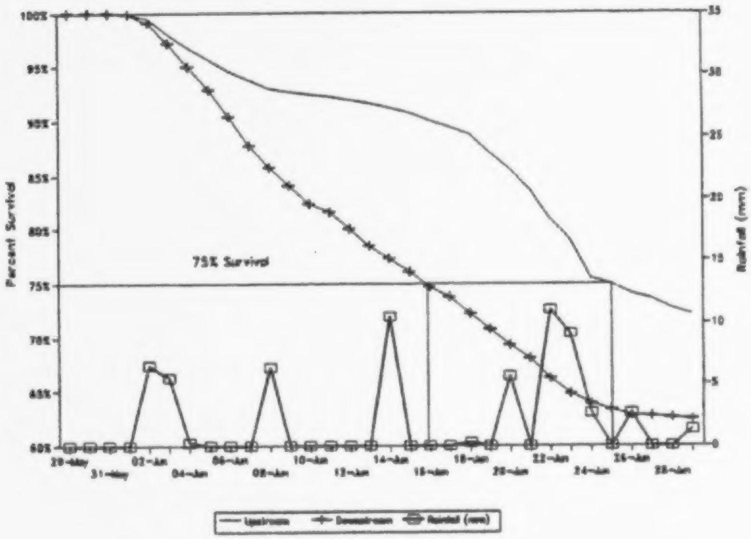

Figure 5. Overall 1990 Rainbow Trout Upstream/Downstream Egg Survival Results with Plotted Rainfall Data and LT25 (Time to 75% Survival) Extrapolations.

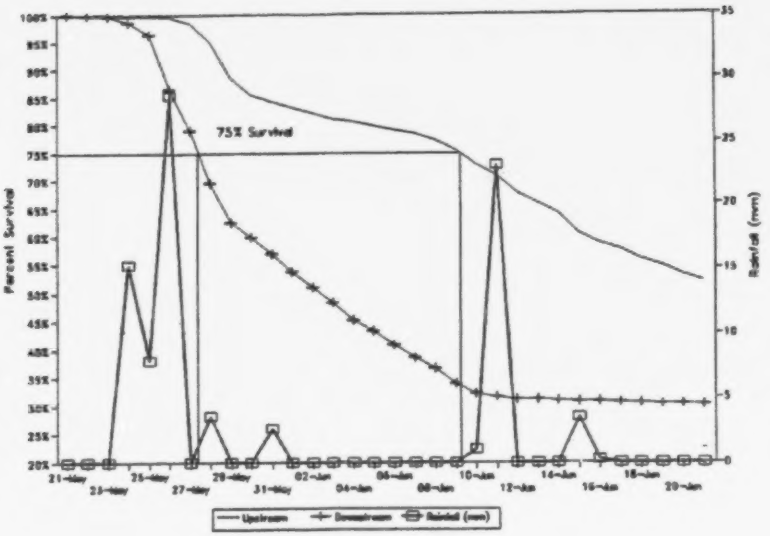

Figure 6. Overall 1991 Rainbow Trout Upstream/Downstream Egg Survival Results with Plotted Rainfall Data and LT25 (Time to 75% Survival) Extrapolations

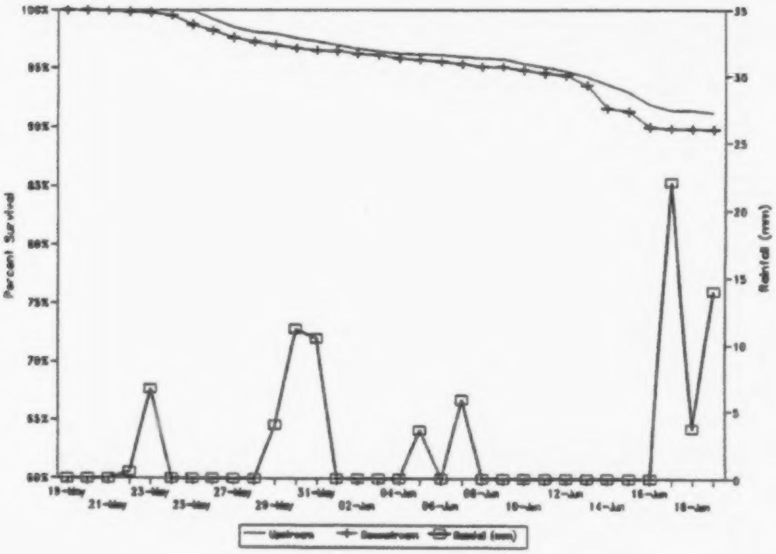

Figure 7. Overall 1992 Rainbow Trout Upstream/Downstream Egg Survival Results with Plotted Rainfall

Based on analysis of variance, each year a significant difference existed in egg survival between stations. The eggs raised at the upstream station showed better survival, in statistical analysis, than the downstream counterparts in 1989, 1990 and 1991. Survival was better at the upstream station in 1992 but as no statistical analysis has been completed, at the time of writing this paper, on the data set it is not known if this difference was significant. In 1990 and 1991 increased survival favoured the upstream eggs throughout the experiment. In 1989, the downstream eggs survived better than the upstream eggs until the final days of the experiment. This was not reflected in the statistical analysis. It was important therefore to examine an alternative survival indicator.

Survival was also measured as the difference in the rate of egg development under various treatments. This analysis compared the length of time, LT25, required to reach 25% mortality. The longer time required to reach the LT25 was an indication of better water quality. The LT25 values are summarized in Table 5 and Figures 4, 5, & 6.

Table 5. Summary of LT25 Values

Treatment	LT25 Values, Days		
	1989	1990	1991
Upstream Overall	24.8	26.9	20.0
Downstream Overall	24.8	17.8	7.2

As measured by LT25, no difference in survival of eggs was detected in 1989 between the upstream and downstream groups. Although the percent survival demonstrated that the downstream eggs did not survive as well as the upstream eggs, the LT25 confirmed that this difference was slight. In 1990 eggs at the upstream location took 9 days more than the eggs at the downstream location to reach 25% mortality. In 1991 the upstream eggs required 13 days more than the downstream eggs to reach this level. From 1989-1991 there was a progressive decrease in LT25 values for the downstream treatments. The upstream treatments did not exhibit any trend over the years studied.

Preliminary survival data for 1992 have been compiled but not analyzed statistically. The egg survival was the best in any study year to date. A slightly greater survival was observed in the eggs at the upstream station. In 1992 a LT25 could not be calculated as 25% mortality was never recorded in any of the treatment groups at either station because of excellent survival of Rainbow Trout eggs.

During the 1989 and 1990 studies filtering the river water had no effect on egg survival. In 1991 eggs raised under filtered conditions had better survival than eggs raised under unfiltered conditions. When the data were compared, filtering of the water had no effect on egg survival. Preliminary 1992 results, although not statistically analyzed, showed that better egg survival occurred in the unfiltered water. When all the data were compared, filtering of river water had no effect on egg survival suggesting that the filtered material had no effect.

The biological program was supported by continuous on-line chemical testing of the St. Clair River. Readings from Lambton Industrial Society's on-line analyzer, during routine and spill event monitoring, did not show contaminant levels that could have been responsible for the lower egg survival at the downstream station in 1990 survival during the blastopore stage of development (Days 5,6,7). This stage has been documented as the most critical stage of Rainbow Trout egg development (Piper et al., 1983). In 1989 and 1992 no rainfall events coincided with decreases in egg survival.

In 1992, a possible rainfall effect was investigated further with immunoassays conducted to quantify pesticide concentrations. Samples collected during dry weather conditions showed non-detectable levels of 2, 4-D and Metolachor at both sampled locations. Atrazine was found at 0.06-0.08 ppb at the upstream site during both wet and dry conditions. Slightly elevated levels, 0.10-0.32 ppb, of Atrazine and Metolachor were found at the downstream location during wet weather sampling. 2, 4-D was not detected in any of the samples. Detected levels of Metolachor and Atrazine, during wet and dry periods, were below the Canadian Water Quality Guidelines for the Protection of Aquatic Life but above the Ontario Ministry of the Environment's Interim Maximum Acceptable Concentrations of 0.05 ppb for Metolachor and 0.06 ppb for Atrazine.

CONCLUSIONS

A multi-species assessment of water quality, that monitored sublethal and chronic endpoints, was conducted at St. Clair River locations upstream and downstream of a heavily industrialized area. Different species can react very differently to the same water quality. This paper emphasizes the results of the Rainbow Trout egg studies.

- *Daphnia magna* and *Daphnia pulex* survival and reproductive rates were studied. Fecundity rates were monitored in Fathead Minnows (*Pimephales promelas*). These evaluations did not detect any difference in the experimental endpoints between the upstream and downstream stations.

- Rainbow Trout eggs were determined to be the most sensitive test material. A significant difference existed each year in survival between stations. The eggs raised at the upstream station had better survival than the downstream counterparts.

- The LT25 was the exposure time required to reach 25% mortality in the sample population of Rainbow Trout eggs. This measure of survival did not always detect a difference in LT25 values between stations in 1989. During 1990 the eggs at the upstream station required 9 days more than the eggs at the downstream station to reach the LT25, and during 1991 the upstream eggs required 13 days more. The longer time required to reach the LT25 was an indication of better water quality.

- Preliminary 1992 results showed the best survival of eggs for any of the years studied. The survival exceeded 85%, so a LT25 could not be calculated.

- The green and eyed stages of egg development were more sensitive to water quality than the hatched stage in the experiments conducted.

- When all the data were compared, filtering of river water had no effect on egg survival suggesting that filtered material had no effect.

- It was speculated from the 1990 and 1991 data that periods of intense rainfall may have had an effect on egg survival particularly at the downstream station. No possible correlation was evident in 1989 and 1992.

- The analysis of multi-species biomonitoring results indicates that yearly events and not trends are being evaluated in the St. Clair River. The results of the Rainbow Trout egg studies support this with comparable survival recorded in 1990 and 1991 and yet better in 1989 and 1992.

ACKNOWLEDGMENT

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PREFERENCE-AVOIDANCE TESTING: TOWARDS GREATER ECOLOGICAL REALISM.

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ABSTRACT

Few studies have examined how the preference-avoidance responses of fish to contaminants may be altered by the presence of other movement-directing factors. To illustrate how such factors can influence responses, we present results from studies investigating the effect of Cd pre-exposure, and shoaling behaviour on the preference-avoidance responses of lake whitefish (*Coregonus clupeaformis*) to Cd. For the pre-exposure experiment, fish were exposed to Cd concentrations of 0, 0.2, 1, or 5 µg/L for three weeks. Then their preference-avoidance responses to Cd were tested in a countercurrent-type trough with clean water entering at one end and water containing sequentially increasing Cd concentrations (0-25 µg/L) at the other. Fish pre-exposed to 1 and 5 µg/L were attracted to test solutions of the same concentration to which they had been exposed previously, while naive (not previously exposed) fish were attracted to 25 µg/L solutions only. This suggests that whitefish can become familiarized to Cd-contaminated water, subsequently preferring it to clean water. For the shoaling experiment, the preference-avoidance responses of naive solitary fish were compared to those of individuals within shoals of four fish. Responses to Cd (0-125 µg/L) were tested in the same manner as before. Individuals in shoals had a lower response threshold and responded more strongly to Cd than did solitary fish. Movements of the shoal appear to be directed by those of its most sensitive members through social facilitation. Previous exposure and social interaction are two environmental factors which can significantly modify the preference-avoidance responses of whitefish to Cd.

EXTENDED SUMMARY

While there is considerable literature on preference-avoidance responses of fish to pollutants, studies are typically restricted to measuring the responses of naive (not previously exposed) fish under very simplified, environmental conditions. In nature, a fish will not encounter contaminant gradients in isolation; rather, it will be simultaneously subjected to several natural physical, chemical or biological stimuli which will also influence the direction in which it chooses to swim. In addition, it may have previously been exposed to the same contaminant resulting in chemosensory impairment, sensitization, desensitization, or familiarization. Consequently, fish may not react to contaminant gradients as predicted by the usual laboratory avoidance tests. Yet there is little information on how the presence of natural movement directing influences or exposure history can modify avoidance responses to contaminants. To illustrate how important these factors can be, we show how pre-exposure to Cd, and shoaling behaviour alter the preference-avoidance responses of lake whitefish (*Coregonis clupeaformis*) to Cd.

Testing was carried out in a countercurrent-type avoidance trough (Scherer and Nowak 1973), where clean water entered at one end and Cd solutions at the other. Water exited the trough via centre drains, resulting in a steep Cd gradient at the centre. For the pre-exposure experiment, whitefish were exposed to Cd for three weeks in flow-through tanks at one of four concentrations: 0 (naive), 0.2, 1 and 5 µg/L. For testing, individual fish were then placed into the trough and allowed 30 min habituation. Following a 15 min pretest where clean water flowed into both ends, Cd was added to one end at concentrations of 0.1, 0.2, 1, 5 and 25 µg/L, which were delivered sequentially at 15 min intervals. During the pretest and each of the five Cd tests, the movements of the fish were tracked manually with a gunsight-type pointer, and the position coordinates automatically digitized and stored on computer every 1 s (Scherer et al. 1989; 1992). For the shoaling experiment, responses to test concentrations of 0.2 - 125 µg Cd/L were compared between solitary whitefish, and individuals within shoals of four fish. Testing was conducted in the same manner as before; the movements of either a solitary fish, or a randomly chosen individual within a shoal were monitored for all six test concentrations.

Results showed that while naive fish were weakly attracted to the highest test concentration only, those pre-exposed to 5 µg/L showed a pronounced attraction to water containing 5 µg/L ($p < 0.05$); similarly, fish pre-exposed to 1 µg/L were attracted to 1 µg/L test solutions, though less strongly. This attraction was also evident in plots of depth of penetration into the untreated and treated sides of the trough; 5 µg/L pre-exposed fish tended to move deeply into the treated side (attraction) then only shallowly penetrating the untreated side before returning to the Cd-contaminated side. This indicates that fish can become familiarized to the odour or flavour that Cd imparts, to the point where they will prefer it to uncontaminated water. This has also been observed for fish pre-exposed to chromium (Anestis and Neufeld 1986), metal mixtures (Hartwell et al. 1987a,b), and bleached kraft mill effluent (Myllyvirta and Vuorinen 1989), suggesting that this phenomenon may apply to a variety of contaminants.

Similar to a previous study (McNicol and Scherer 1991), whitefish in the shoaling experiment displayed a bimodal dose-response relationship with Cd. Both solitary fish and individuals within a shoal avoided low (0.2-1 µg/L) and high (≥11 µg/L) concentrations while being unresponsive to intermediate levels. However shoal individuals were more sensitive to contact with Cd than were solitary fish. While shoal individuals more strongly ($p = 0.052$) avoided higher [Cd], the greater sensitivity was more clearly illustrated by differences in depth of penetration into the untreated side of the trough. Shoal fish retreated more deeply into this side after contact with Cd than did solitary fish, both at low and again at high test concentrations ($p = 0.025$). Our results suggest that shoaling fish react to the responses of the most sensitive individuals within a shoal. Thus, just as the "many eyes" within a shoal increase an individual's sensitivity to a predator's approach through social transmission of startle responses (Magurran et al. 1985; Godin et al. 1988), "many noses" may increase chemical sensitivity via a similar mechanism.

Prior exposure and social interaction produced opposite changes in the preference-avoidance responses of whitefish to Cd: the former promoted attraction, the latter enhanced avoidance. These observations illustrate how conventional, preference-avoidance testing may not accurately reflect how fish will respond to contaminant gradients in complex, natural settings.

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MITOCHONDRIAL ENZYMES AND MIXED FUNCTION OXIDASES AS BIOINDICATORS OF XENOBIOTIC EXPOSURE IN RAINBOW TROUT.

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ABSTRACT

Industrial spent chemicals often discharged into aquatic ecosystems can be toxic to fish species. To monitor systemic effects of xenobiotic chemicals in fish, studies were conducted to investigate (i) critical analytical characteristics of key mitochondrial enzymes and microsomal mixed function oxidases (MFO), and (ii) *in vitro* and *in vivo* effects of selected xenobiotic chemicals on the activity of these enzymes in hepatic tissues of rainbow trout. Among the enzymes tested, the activities of aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin-O-deethylase (ECOD), and mitochondrial coupling of succinate oxidation and phosphorylation (ADP:O) were markedly sensitive to increased assay temperatures. Direct (*in vitro*) treatment of isolated mitochondria with chlorinated organic compounds showed that (i) 2,4-dichlorophenol and dichloromaleic anhydride caused marked stimulation of ATP-ase, uncoupling of oxidative phosphorylation and inhibition of ADP-dependent oxidation of succinate, and (ii) 2,6-dichlorobenzene and chloroform inhibited succinate oxidation both in the presence and absence of ADP. Treatment of rainbow trout with 3-methylcholanthrene (3-MC) and phenobarbital (PB) showed that only 3-MC produced characteristic induction of AHH, ECOD and cytochrome P-450 (P-450); other biochemical parameters were not altered. The (*in vitro*) inhibition of the induced form of AHH specifically by α-naphthoflavone would also indicate that the 3-MC specific isozymic form(s) of P-450 was induced in the treated fish.

The discharge of industrial and municipal effluents into the aquatic environment can be deleterious to fish and other species. Because the chemicals present in these effluents undergo dilution downstream from the source, it is difficult to assess pollutant-related systemic biological changes in resident fish species. In fish species, the systemic effects caused by a number of water-borne organic chemicals (e.g. chlorinated biphenyls, polyaromatic hydrocarbons, various chlorinated aliphatic and aromatic compounds) are now assessed by conducting biochemical tests as early warning indicators. Studies have shown that among enzymes involved in xenobiotic metabolism, cytochrome P-450 (P-450) and P-450 linked mixed function oxidases (MFO) are markedly induced in fish exposed to water contaminated with industrial pollutants (Payne et al. 1987). Other more serious physiological impairments (e.g. mitochondrial dysfunction) could also occur depending upon the chemicals present in contaminated waters.

Because the procedures for a number of biochemical parameters used in this study have not been tested specifically in fish species, it was necessary to optimize and standardize these tests. In this paper we have provided related information for (i) key metabolic enzymes of mitochondria, and (ii) P-450 linked MFO activities in rainbow trout liver. Furthermore, we have assessed the (*in vitro*) sensitivity of mitochondrial enzymes to several chlorinated organic compounds, and the response of various biochemical activities in liver of fish exposed to two model inducers of MFO, 3-methylcholanthrene (3-MC) and phenobarbital (PB).

Materials and Methods

Experimental Fish

Mature rainbow trout (*Oncorhynchus mykiss*), weighing >250 g, were used in all studies. The fish were reared at the Aquatic Biology facility of the Alberta Environmental Centre. Animal care and maintenance were performed according to standardized procedures. All experimental procedures were conducted according to Canadian Council of Animal Care guidelines (CCAC 1984). For all (*in vitro*) studies the control (untreated) fish were euthanized by blunt cranial trauma producing immediate unconsciousness, and the excised liver tissues were used to isolate subcellular fractions.

Exposure Studies

Fish were injected intraperitoneally with a single dose of 3-MC (12.5 mg/kg body weight) in corn oil or PB (100 mg/kg body weight) in 0.9% NaCl solution. Control fish were injected with an equivalent volume of vehicle solution alone (corn oil or 0.9% NaCl solution). The fish were euthanized as mentioned above at 96 hours post injection and liver tissues were immediately excised to prepare subcellular fractions.

Subcellular Fractionation and Enzyme Assays

Excised liver tissues were washed with cold 0.9% NaCl solution. These samples were then processed and fractionated into mitochondrial, microsomal and cytosolic fractions by standardized procedures (Schuler and Khan 1991).

All enzyme assays were carried out under limiting concentrations of enzyme source under optimal assay conditions. Appropriate blanks for each enzyme were carried out simultaneously. The activities of 7-ethoxycoumarin-O-deethylase (ECOD), aryl hydrocarbon hydroxylase (AHH), NADPH-cytochrome c reductase (NCR), glutathione transferase (GT), and the concentration of P-450 were assayed according to procedures described by Khan et al. (1989). Other enzyme activities were assayed as follows: glucose-6-phosphate dehydrogenase (GDH) (Khan et al. 1987), ATP-ase (Seniuk et al. 1992), succinate oxidation and coupled phosphorylation (George and Khan 1992).

(*In Vitro*) Studies with Chlorinated Organic Compounds

The effects of various chlorinated organic compounds on selected enzyme activities were monitored by directly adding specified concentrations of each test compound to assays containing the enzyme fraction. Enzyme assays without the addition of test chemical served as respective controls.

Data Analyses

All (*in vitro*) experiments were repeated three times with different enzyme preparations, and the trends were found to be highly reproducible. The data in Table 2 were taken from a representative experiment.

For exposure studies, the difference between a treatment and its respective vehicle control was analyzed by one way analysis of variance.

Results

Activities of Mitochondrial, Microsomal and Cytosolic Enzymes in Rainbow Trout Liver

Table 1. Enzyme activities in rainbow trout liver. Values represent mean ± SD of 6-8 fish.

Cellular fraction/enzyme	Activity or concentration (units/mg protein)
Mitochondrial	
SO$_x$ State 3 (+ ADP)*	21 ± 7
SO$_x$ State 4 (- ADP)*	6 ± 1
ATP-ase*	146 ± 15
Microsomal	
P-450**	167 ± 48
AHH^{+}	1.7 ± 0.5
ECOD^{+}	5.6 ± 0.7
NCR*	51.4 ± 14.0
Cytosolic	
GDH*	481 ± 115
GT*	271 ± 67

*mmol/min; ** pmol; $^{+}$ pmol/min

Hepatic activities of enzymes assayed with isolated subcellular fractions are shown in Table 1. These values are from control fish, and are measured under conditions optimized for each enzyme's assay. Among these enzymes, the activity of (i) AHH and ECOD was maximal at 18°C and decreased markedly at 37°C; (ii) mitochondrial succinate oxidation (succinate oxidase; SO_x) showed good coupling with phosphorylation (ADP:O = 1.5-2.1) up to 25°C, but at higher temperatures (e.g. 37°C) this coupling was lost (Figure 1); and (iii) other enzymes were higher at 37°C than at lower temperatures.

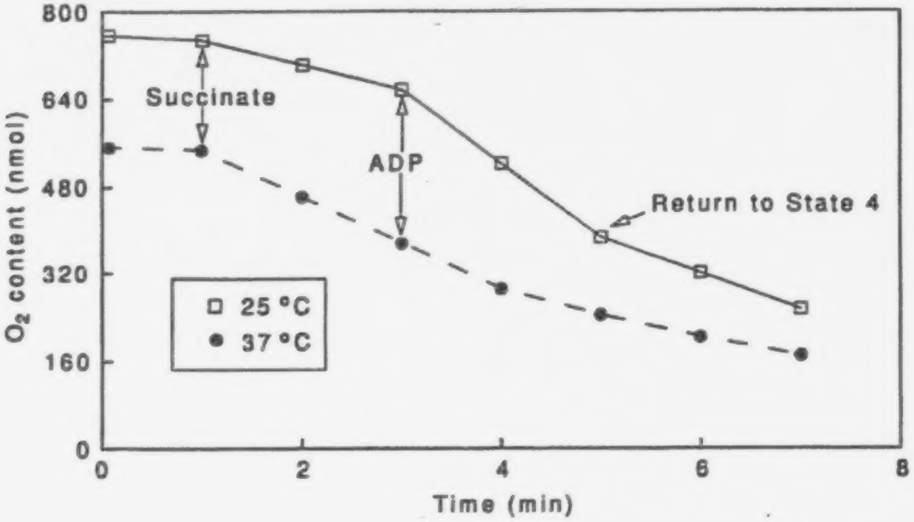

Figure 1. Effect of assay temperature on ADP-dependent (State 3) SO_x activity.

Direct (*In Vitro*) Effects of Selected Chlorinated Organic Compounds on Mitochondrial Enzyme Activities

Table 2. Effects of DCP on mitochondrial succinate oxidation and coupled phosphorylation.

DCP (µM)	Succinate Oxidation (nmol/min/mg protein) State 3	Succinate Oxidation (nmol/min/mg protein) State 4	Phosphorylation (ADP:O)
0	29.3	9.2	2.1
165	32.2	12.9	1.8
300	23.9	18.4	0.0

Addition of 2,6-dichlorophenol (DCP) caused a marked stimulation of SO_x activity under non-phosphorylating (State 4; -ADP) conditions. The oxidation rate under phosphorylating (State 3; +ADP) conditions was also altered, but the effects were less marked. Addition of DCP caused concentration-dependent impairment of oxidative phosphorylation (ADP:O). The activity of mitochondrial ATP-ase was markedly stimulated (>50%) upon treatment with DCP (0.5 - 1.0 µM). Stimulation of ATP-ase was also observed with dichloromaleic anhydride (DCMA) treatment.

Other chlorinated compounds (e.g. chloroform, DCMA, 2,6-dichlorobenzene) caused marked and concentration-dependent inhibition of SO_x under both phosphorylating and nonphosphorylating states.

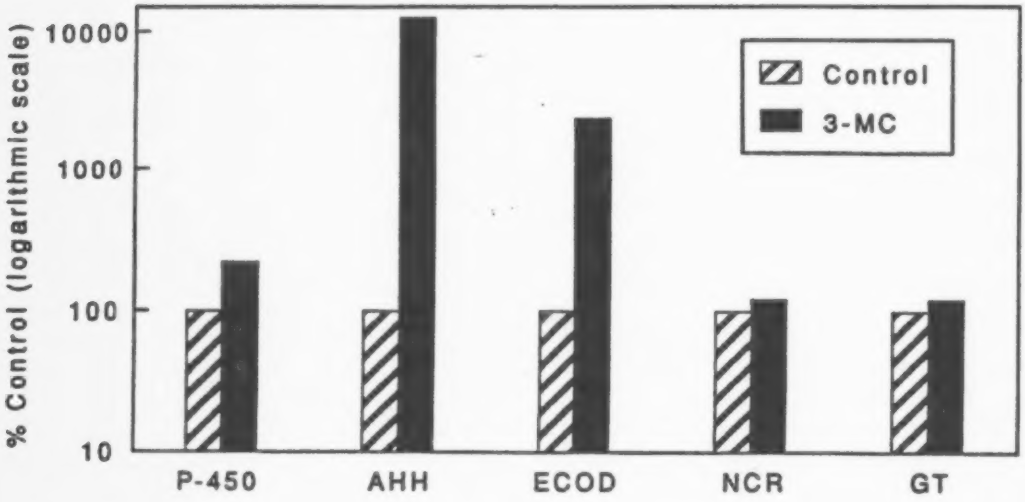

Figure 2. Effect of 3-MC exposure on enzyme activities.

Fish treated with 3-MC showed marked increases from control fish in the content of P-450 and in the activities of AHH and ECOD in hepatic microsomes. Among these microsomal indices AHH was the most sensitive to 3-MC treatment. Fish treated with PB did not show any significant change from control fish in these hepatic biochemical parameters. Additional (*in vitro*) experiments with microsomes from 3-MC treated fish showed highly specific sensitivity of AHH activity to α-naphthoflavone.

Discussion

This paper provides control (baseline) values of several key enzymes in hepatic tissues of mature rainbow trout (Table 1). The enzymes selected for study are physiologically important for (i) cellular energy homeostasis (e.g. mitochondrial enzymes), and (ii) metabolism of xenobiotic and endogenous substances (e.g. MFO, NCR, GT, GDH). Although considerable information is available about MFO activities in various fish species (Payne et al. 1987; Smith et al. 1991) relatively little is known about the biochemical characteristics of mitochondrial processes. Even for MFO there is a need to develop critical guidelines for proper standardization of assay conditions and other analytical aspects in order to use these tests in regulatory assessment of health and exposure effects.

The isolation of functionally intact mitochondria for *in vitro* and *in vivo* assessment of diagnostic effects is often technically difficult. Results presented in this paper showed that our isolation procedure provided functionally intact mitochondrial preparations as evidenced by their good respiratory control ratio (state 3/state 4 rates of SO_x) and excellent coupling of oxidative phosphorylation (ADP:O = 1.5-2.1). This coupling of oxidative phosphorylation was severely impaired at 37°C and this could be due to a marked increase of ATP-ase activity at this temperature. Such biochemical events *in vivo* would cause severe health impairments in affected fish.

Chlorinated organic compounds (e.g. chlorophenols and chloroform) are found ubiquitously in contaminated aquatic environments. The results presented in this paper showed that direct (*in vitro*) interaction of DCP with rainbow trout mitochondria caused a marked uncoupling effect on oxidative phosphorylation and stimulation of ATP-ase activity. Other chlorinated compounds (e.g. chloroform, DCMA, 2,4-dichlorobenzene) inhibited SO_x by altering either the lipophilic membrane constituents or the electron transport system. Because of the uncoupling effect on oxidative phosphorylation, the chlorophenols are considered a threat to a wide variety of aquatic organisms (Ahlborg and Thunberg 1980). As chlorinated phenolics (e.g. chloroguaiacols and chlorocatechols) often present in bleach liquors of kraft pulp mills are shown to accumulate in fish exposed to pulp mill effluents (Ahlborg and Thunberg 1980), the use of mitochondrial biomarkers for health assessment can be particularly useful.

The induction of MFO as an early warning assessment tool was found to be particularly useful in rainbow trout exposed to a polyaromatic hydrocarbon type chemical (3-MC); however, the trout exposed to an anesthetic type chemical (PB) failed to induce any MFO activity. This indicates that more research is needed to find new biochemical markers for such types of xenobiotic substances.

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CYTOCHROME P-450 RELATED ENZYMATIC ACTIVITIES IN FISH FROM WESTERN CANADIAN RIVERS RECEIVING CHLORINATED KRAFT PULP MILL WASTES.

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Fish downstream from sources of bleached kraft mill effluent have been taken from several river locations for comparison with fish from upstream "control" sites. Juvenile chinook salmon downstream of mill sites at Prince George and Quesnel were taken in the spring of 1988 for comparison with similar "control" fish. The results showed increases in post-mitochondrial supernatant ethoxyresorufin-O-deethylase activities of up to about fifty-fold in the downstream fish, and correlated well with chlorinated dibenzodioxin and dibenzofuran residues. Similar high values were obtained from a few mountain whitefish taken at locations downstream from the effluent sources. More recently, mountain whitefish have been sampled from two Canadian locations in the Columbia system. One location, was downstream from the mill at Castlegar, and a second was upstream at Brilliant Reservoir on the Kootenay River. Again, fish from the downstream site displayed striking elevations in several cytochrome P-450-related measurements and again there was a striking correlation to chlorinated/dioxin furan residues. Other "control" collections of mountain whitefish from Oregon had low activities, as did a series of collections for whitefishes of several species from western and northern Canada.

P450IA INDUCTION AND OTHER RESPONSES IN MOUNTAIN WHITEFISH EXPOSED TO BLEACHED KRAFT MILL EFFLUENT (BKME) IN NORTHERN ALBERTA.

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Mountain whitefish (Prosopium williamsoni), MTWT exhibit the highest body burdens of compounds such as EOC1 and dioxin reported in Western Canadian fish exposed to BKME. Therefore, to assess fish health, numerous biomarker responses were measured over several seasons in MTWT from a reference stream or exposed to biologically-treated effluent from a Northern Alberta pulp mill. Liver and gonadal somatic indices and condition factors, as well as histopathological lesions and parasite loadings, were similar for fish from both systems. P4501A activity (ethoxyresorufin O-deethylase, EROD) was induced in mill site MTWT 25-fold over reference fish in spring 1991. Autumn mill site EROD values were lower, possibly due to cooler ambient water or sexual maturation, or to fewer available inducers due to mill process changes. Results of further investigation of these trends in fish collected in spring 1992 will be reported. While MTWT are exposed to BKME from the mill for substantial periods, P4501A induction is the only biological response observed to date, and does not appear to be linked to adverse population or individual health effects.

IN VIVO AND _IN VITRO_ EROD INDUCTION IN FISH BY DIOXINS AND FURANS. J.L. Parrott[1,3], J.H. Clemons[1], P.V. Hodson[2], N.C. Bols[1] and D.G. Dixon[1]. [1]Dept. of Biology, Univ. Waterloo, Waterloo, ON; [2]NWRI, Environment Canada, Burlington, ON; [3]Dept. Fisheries and Oceans, Burlington, ON.

We tested the potencies of five polychlorinated dibenzo-*p*-dioxins (PCDDs) and four polychlorinated dibenzofurans (PCDFs) for inducing liver ethoxyresorufin-O-deethylase (EROD) activity in rainbow trout (*Oncorhynchus mykiss*) and in a rainbow trout liver cell line, RTL-W1. Single oral doses of 2,3,7,8-tetra (2,3,7,8-TCDD; 0.06-2 $\mu g\ kg^{-1}$), 1,2,3,7,8-penta (1,2,3,7,8-PnCDD; 0.03-10 $\mu g\ kg^{-1}$), 1,2,3,6,7,8-hexa (1,2,3,6,7,8-HxCDD; 0.3-8 $\mu g\ kg^{-1}$), 1,2,3,4,7,8-hexa (1,2,3,4,7,8-HxCDD; 0.1-10 $\mu g\ kg^{-1}$) or 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1,2,3,4,6,7,8-HpCDD; 0.3-80 $\mu g\ kg^{-1}$), 2,3,7,8-tetra (2,3,7,8-TCDF; 0.79-25 $\mu g\ kg^{-1}$), 1,2,3,7,8-penta (1,2,3,7,8-PnCDF; 0.79-25 $\mu g\ kg^{-1}$), 2,3,4,7,8-penta (2,3,4,7,8-PnCDF; 0.39-12 $\mu g\ kg^{-1}$) or 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF; 0.28-8.6 $\mu g\ kg^{-1}$) given to live trout elevated liver EROD activity in a dose-responsive manner after eight days. Three-day incubations of the RTL-W1 cell line with these same congeners generated similar dose-response curves. The concentrations used in the RTL-W1 assay were: 2.5-158 pM 2,3,7,8-TCDD, 1-195 pM 1,2,3,7,8-PnCDD, 128-770 pM 1,2,3,6,7,8-HxCDD, 6.7-40.7 pM 1,2,3,4,7,8-HxCDD, 83.1-499 pM 1,2,3,4,6,7,8-HpCDD, 10-299 pM 2,3,7,8-TCDF, 30-977 pM 1,2,3,7,8-PnCDF, 8.7-208 pM 2,3,4,7,8-PnCDF, or 9.3-149 pM 1,2,3,4,7,8-HxCDF.

Generally, the literature shows that in mammals 2,3,7,8-TCDD is the most toxic of the PCDDs and PCDFs. The potencies of other less toxic congeners are related to the potency of 2,3,7,8-TCDD using toxic equivalent factors (TEFs). A TEF is a fraction describing congener X's potency (the amount of 2,3,7,8-TCDD needed to cause a certain effect, divided by the amount of congener X needed to cause the same effect).

We found, in both live trout and RTL-W1 cells, two congeners tested, 1,2,3,7,8-PnCDD and 2,3,4,7,8-PnCDF, were more potent than 2,3,7,8-TCDD (Table 1). The TEF for live trout calculated for 1,2,3,7,8-PnCDD (1.2) was based on the oral dose given to fish. This dose approximation is not as accurate as liver concentration, as many additional factors come into play with oral dose, which cause greater variability in the EROD response in fish. However, the TEF for 1,2,3,7,8-PnCDD in the RTL-W1 cell line (1.4) agrees with that calculated using the oral dose in fish, and so supports the conclusion that 1,2,3,7,8-PnCDD is a more potent inducer of EROD than 2,3,7,8-TCDD. For the TEF for 2,3,4,7,8-PnCDF in live trout, liver concentrations of this congener were compared to liver concentrations of 2,3,7,8-TCDD eliciting EROD activity significantly above control levels. The TEFs in live trout represent for the most part the "worst case" scenario, as concentrations of congeners are the closest near the threshold for EROD, and farther apart at maximal EROD activities. In calculation of TEFs in live trout, we chose to use the threshold EROD concentrations, which result in the highest TEF and thus afford the most protection to fish. In the RTL-W1 cells, TEFs were calculated conventionally, comparing the plate concentrations that caused 50 % of maximal EROD activity.

For the PCDDs tested, TEFs were very similar for live trout and RTL-W1 cells. 1,2,3,7,8-PnCDD was the most potent inducer in both (TEF=1.2-1.4), followed by 2,3,7,8-TCDD with a TEF of 1, by definition. Of the two HxCDDs, 1,2,3,4,7,8-HxCDD was more potent, with a TEF of 0.52 in trout and 0.76 in cells, compared to 1,2,3,6,7,8-HxCDD, that had a TEF of 0.37 in trout and 0.13 in cells. 1,2,3,4,6,7,8-HpCDD was the least potent inducer in live trout, with a TEF of 0.036, while the TEF for RTL-W1 cells was 0.15.

For the PCDFs, TEFs were again similar for live trout and for the RTL-W1 cell line, and the ranking of potencies was the same. The most potent was 2,3,4,7,8-PnCDF (TEF=5.3 in trout, 1.2 in cells), followed in order by 1,2,3,4,7,8-HxCDF (TEF=0.47 in trout, 0.97 in cells), 2,3,7,8-TCDF (TEF=0.25-0.28) and 1,2,3,7,8-PnCDF (TEF=0.24 in trout, 0.13 in cells).

Table 1. Toxic equivalent factors for induction of EROD activity in live rainbow trout, RTL-W1 cells and international TEFs based largely on mammalian data.

CONGENER	TOXIC EQUIVALENT FACTORS		
	RAINBOW TROUT[a]	RTL-W1 CELLS[b]	INTERNATIONAL[c]
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PnCDD	1.2[d]	1.4	0.5
1,2,3,6,7,8-HxCDD	0.37[d]	0.13	0.1
1,2,3,4,7,8-HxCDD	0.52	0.76	0.1
1,2,3,4,6,7,8-HpCDD	0.036	0.15	0.01
2,3,7,8-TCDF	0.28	0.25	0.1
1,2,3,7,8-PnCDF	0.24	0.13	0.05
2,3,4,7,8-PnCDF	5.3	1.2	0.5
1,2,3,4,7,8-HxCDF	0.47	0.97	0.1

a. TEFs calculated based on liver concentrations necessary to elevate EROD significantly ($p=0.05$) above control values.
b. TEFs calculated based on plate concentrations necessary to elevate EROD to 50 % of maximal level (EC50).
c. International TEFs from NATO/CCMS, Report 178, 1988.
d. TEFs calculated based on oral dose necessary to elevate EROD significantly ($p=0.05$) above control values.

The ranking of potencies of PCDDs and PCDFs in trout and cells was similar to the ranking based on mammalian data. However, most of the TEFs in live trout and in the rainbow trout liver cell line were larger than the mammalian-based international TEFs (Table 1). Hence, risks to fish of PCDDs and PCDFs may not be adequately predicted by mammalian-derived TEFs.

ISOLATION OF *EROD* INDUCING MATERIAL FROM PRIMARY AND SECONDARY TREATED KRAFT MILL EFFLUENTS.

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ABSTRACT

Induction of hepatic biotransformation enzymes for fish in waters receiving effluents from pulp mills have received much recent attention but the responsible compound(s) remain unidentified. Effluent fractionations employing nanofiltration were performed on primary effluent and the corresponding secondary effluent from a modernized bleached kraft mill. Using induction of liver ethoxyresorufin-o-deethylase (EROD) as the indicator of activity, rainbow trout (*Salmo gairdneri* R.) were exposed in static, non-renewed, 96 hour waterborne regimes. Fish were exposed to undiluted whole effluent, filtered effluent (<1µm), filtered solids resuspended in laboratory water, low molecular weight (<400d) and high molecular weight (>400d) fractions. The induction potential of whole effluent remained unchanged after conventional secondary treatment or filtration. The breakdown of EROD activity among the fractions was the same for both types of effluents. For both effluents, virtually all activity was lost after nanofiltration. Chemical characterizations of active and inactive fractions have thus far revealed no correlations between induction and bulk parameters such as AOX (adsorbable organic halogens), DOC (dissolved organic carbon) or measured levels of resin, fatty and bacterial acids.

MATERIALS AND METHODS

Nanofiltration

Effluents (400L) were obtained from a modernized bleached kraft mill employing both primary treatment (clarifier followed by settling basin, 12 hour hydraulic retention time) and secondary treatment (aerated lagoon, 5.5 day hydraulic retention time). Effluents were immediately fractionated upon arrival to the laboratory using nanofiltration with a nominal molecular size cutoff of 400 daltons (Filmtec NF40-40, Minneapolis, MN). Prior to nanofiltration, effluents were filtered via a two step process consisting of continuous flow centrifugation (10,000 rpm, 2L/min) and pressure filtration through 1µm glass fibre filters (Gelman Sciences, Rexdale, ON). The high molecular size fraction was concentrated to approximately 10L and was purified by dialysing with 8 X 100L distilled water. For fish exposures, this fraction was rediluted in laboratory water to match the colour of whole effluent. Based on recovery from filtration, solids were also rediluted in laboratory water to their estimated effluent proportions.

Fish Exposures and EROD Determinations

Immature rainbow trout (*Salmo gairdneri* R.) were obtained from Rainbow Springs Hatchery, Thamesford, Ontario and acclimated for at least 10d. Fish (5-10g) were exposed in aerated, static, waterborne regimes to undiluted fractions and whole effluents. Exposures were conducted at a loading rate of approximately 5g/L, maintained at 15°C in the dark; feeding was stopped 48h prior to exposures. After 96h, fish were sacrificed, and wet and liver weights were taken. Livers (<0.25g) were immediately homogenized in cold HEPES (Sigma Chemical Co., St. Louis, MO) buffer (0.02M, pH 7.5) and centrifuged at 11,500rpm for 20 min at 2°C. Supernatants were drawn off and stored at -80°C until assayed for EROD activity. For each fish, three sample replicates and one blank assay were performed. 100µl aliquots of microsomal suspension were placed into tubes containing 1250µL HEPES buffer (0.1M, pH 7.8), 10µL 0.154mg/mL $MgSO_4$ (Anachemia, Toronto, ON), 50µL 40mg/mL Bovine Serum Albumin (Sigma Chemical Co.) and 30µL 20mg/mL NADPH (Sigma Chemical Co.). Tubes were placed in a 25°C water bath and 20µL of 7-ethoxyresorufin (Sigma Chemical Co.) solution (0.03mg/mL DMSO) added. The ensuing production of resorufin was stopped after exactly 12 minutes by the addition of 3mL of methanol (DIG grade, Caledon Laboratories, Georgetown, Ontario). Methanol was added prior to ethoxyresorufin in blank determinations. After centrifugation (5100rpm for 20 min at 3°C), the fluorescence of the supernatants was read at an excitation wavelength of 530nm and slit width of 2.5mm and an emission wavelength of 585nm and slit width of 20mm on a Perkin Elmer LS50 Spectrometer. EROD activity was determined by interpolation against a resorufin standard curve and activities were standardized for protein content using a modified Lowry assay.

Chemical Characterizations of Fractions

Samples taken for each bulk fraction were: AOX (adsorbable organic halogen), DOC (dissolved organic carbon), major ions, and trace organics. AOX samples were preserved by acidification with concentrated nitric acid (Analar grade, Anachemica, Toronto, ON) before analysis on a Mitsibushi TOX-10 organohalogen analyzer. DOC samples were acidified with concentrated sulphuric acid (Analar grade, Anachemia, Toronto, ON) and purged with nitrogen prior to analysis on a Beckman 915B total carbon analyzer. Values were obtained against potassium phthalate standards. Trace organics samples were acidified with concentrated sulphuric acid, 1,3,5-tribromobenzene and 2,4-dibromophenol were spiked as internal standards and 5mL 0.1g/mL L-ascorbic acid (Malinkrodt, St. Louis, MO) solution was added as a phenolic preservative. Finally, 100mL dichloromethane (Burdick & Jackson, Toronto, ON) was added and the samples stirred before storage and analysis. The analytical fractionation can be summarized below (Figure 1):

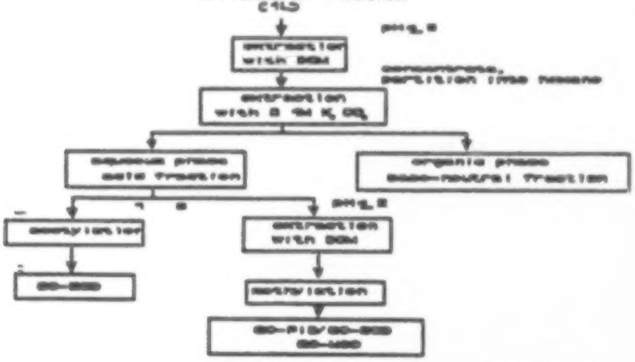

Figure 1. Analytical Fractionation and Analysis

RESULTS

For both primary and secondary effluents, the distribution of EROD induction among the fractions was the same with virtually all induction lost after nanofiltration as shown below with final effluent.

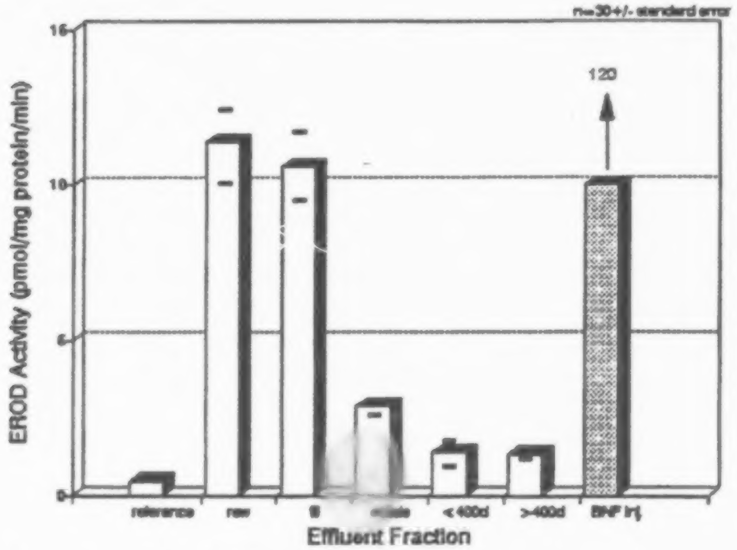

Figure 2 Final Effluent EROD Distribution

There was no change in induction going from primary effluent to the corresponding secondary effluent both for whole effluents and for the <1µm fractions which demonstrated induction (Figure 3). A recombination of final effluent fractions was attempted to determine whether activity was lost as a direct result of nanofiltration, however virtually all EROD activity in the effluent had disappeared during three weeks storage in sealed containers, darkness and 4°C.

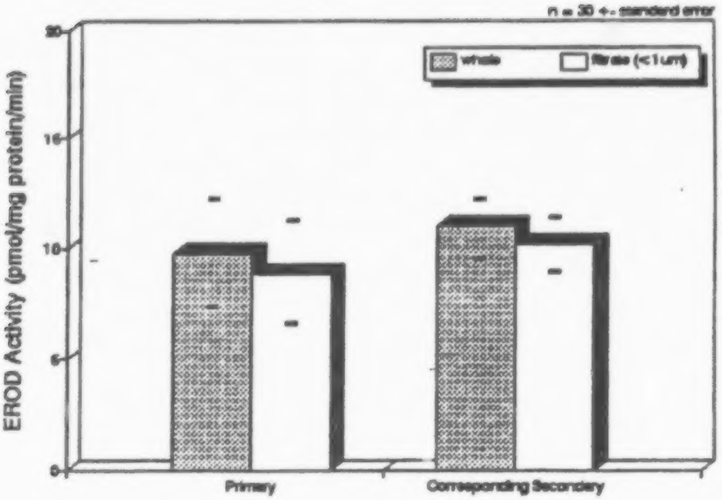

Figure 3 Primary vs. Secondary Induction

Chemical characterizations to date show no correlation between EROD activity and DOC, AOX, or measured levels of strong acids. Analyses are continuing on base neutral fractions.

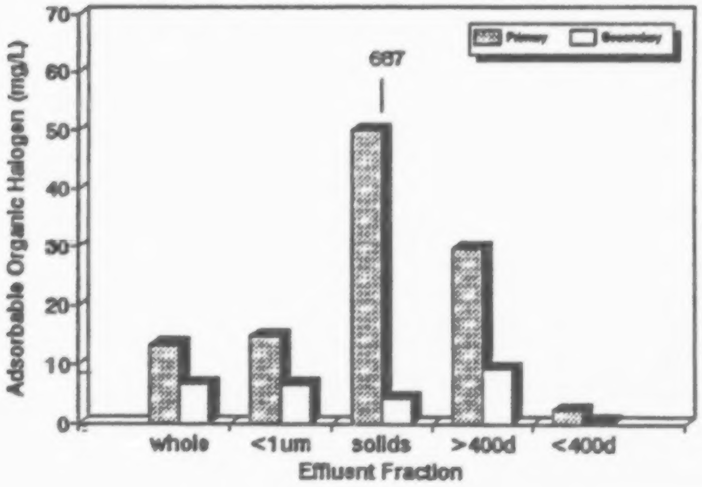

Figure 4 AOX Levels in Fractions used for Trout Exposures

CONCLUSIONS

For the effluents tested, most induction potential was lost after nanofiltration and short term storage. Conventional secondary treatment (5.5 day aeration) did not change the induction observed for primary treated effluent. Induction was isolated to the <1μm water phase prior to nanofiltration. Induction did not correlate with bulk measurements such as DOC, AOX and any of the strong acids measured (resin, fatty and bacterial).

ACKNOWLEDGEMENTS

We wish to thank the following organizations for technical and financial support. Industry, Science and Technology Canada, Environment Canada, Department of Fisheries and Oceans, the Mott Foundation and the Biology Department at the University of Waterloo.

SEASONAL EFFECTS ON HEPATIC MIXED-FUNCTION OXIDASE ENZYMES IN WHITE SUCKERS (*Catostomus commersoni*) FOLLOWING INJECTION WITH PCB CONGENER 77.

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Dose dependency was studied for induction at high and low seasonal levels of the liver microsomal MFO enzymes. The seasonal cycle of EROD and AHH activities in white suckers from a lake in northwestern Ontario was examined. Both activities were lowest for the period just prior to and during spawning early in June. Wild fish were caged in the lake during the spawning period in spring and were given different doses of ^{14}C-PCB congener 77 (3,3',4,4'-tetrachlorobiphenyl) in corn oil by intraperitioneal injection for five days. Similar treatments were also conducted in late September. EROD and AHH induction was noted only at the highest dosage (1000 ug kg $^{-1}$) for both the spring and fall treatments. However, liver residues (from ^{14}C content) indicated a statistically lower induction response by the fall treatments. Livers from the fall, with similar PCB concentrations as the spring samples, had lower induced enzyme activities; possibly this could be attributed to lower water temperatures in the fall. The induced enzyme activities in livers from fall treated fish were still significantly higher than any of the naturally occurring activities recorded throughout the year.

MIXED FUNCTION OXYGENASE CATALYTIC ACTIVITIES AND PCB CONGENER RESIDUES IN A GROUP OF BELUGA WHALES SUBJECT TO STARVATION IN THE WESTERN CANADIAN ARCTIC.

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Some beluga whales entered the freshwaters of the Husky Lakes in the Mackenzie delta and became trapped with the formation of ice. It was clear that the whales could either be harvested or allowed to suffocate, and a decision was taken by local Inuit hunters to harvest the whales. Measurements of whale length and weight indicated that the whales weighed, on average, about 200 kg less than whales of their length would normally weigh. At the time the whales were killed, small samples of liver were placed on dry ice and fast-frozen; they were shipped by air to Winnipeg and maintained at -60°C until analyzed for EROD and AHH enzyme activities and for cytochrome P-450 content. Samples of blubber were also analyzed for organochlorine contaminants including several mono-ortho and non-ortho PCB congeners. Statistically there were very close relationships among the enzymatic measurements and the sum of the PCB congeners (r=0.89 for EROD, 0.87 for AHH, and 0.81 for P-450). In view of the strength of these and other statistical associations, it is hypothesized that mobilization of lipid reserves occurred during the period of starvation and that residues mobilized at the same time may have induced the cytochrome-P-450-associated catalytic activities.

PCB CONGENERS AND ORGANOCHLORINE PESTICIDES IN THE BLOOD OF LAKE ONTARIO FISH CONSUMERS.

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Whole blood samples from 117 Lake Ontario recreational fishermen were collected in 1981. The age and sex of the individuals were collected along with personal estimates of their average fish consumption per year and their average years of fish consumption. The samples were analyzed for PCB congeners and some organochlorine pesticides by capillary gas chromatography with electron capture detection. About 10% of these analyses were confirmed by GC/MS.

A much smaller number of PCB congeners was found in the blood samples compared to the number present in Lake Ontario fish and in PCB Aroclor mixtures. PCB congeners with chlorine substitution in both the 4 and 4' positions were the predominant PCBs in the blood samples. A statistically significant increase in total PCBs in blood was found with increasing person age. There appeared to be an increase in total PCBs with fish consumption but the relationship was not statistically significant.

Hexachlorobenzene and p,p-DDE were detected in every blood sample. Other organochlorines found in some samples were alpha-BHC, octachlorostyrene, pp-DDT, mirex and photomirex.

ACCIDENTAL OXIDATIVE METABOLISM AS A PREDICTOR OF CHRONIC TOXICITY OF XENOBIOTIC ORGANIC COMPOUNDS.

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A Mechanistic Structure Activity Relation (MSAR) will be described, correlating the inducible oxidizing power of the PolySubstrate MonoOxygenase (PSMO) system to the strength of weakest C-H bonds in xenobiotic compounds. The conversion of saturated hydrocarbons to corresponding alcohols is taken as the "intended" behaviour on the "control" structures. "Treated" structures, represented by such changes as chlorination, desaturation, or addition of amine on either groups can either alter the reactivity at the intended carbon atom or present alternative sites for oxidation. These "accidental" reactions usually release free radicals which can lead to disease conditions. If they are released into the aqueous structures of cells, non-cancerous lesions result. If released into the lipid tissues, cancer, mutations, and teratogenicity can occur. Examples of each accidental behaviour will be described.

TOXAPHENE, PCBs, AND DDTs IN LIVERS OF BURBOT FROM THE YUKON RIVER SYSTEM.

J. Eamer, Environment Canada, Environmental Protection, Whitehorse, YK, (403) 667-3402; D.C.G. Muir, Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MN; M. Palmer, Department of Indian and Northern Affairs, Northern Affairs Program, Whitehorse, YK; K. Kidd, University of Alberta, Department of Zoology, Edmonton, AB.

Results of an assessment of Lake Laberge, downstream of Whitehorse, Yukon, on the upper Yukon River system, showed unexpectedly high levels of organochlorines in some fish species. Burbot livers had the highest concentrations of toxaphene, PCBs and DDTs (respective wet weight means of 2740, 1288 and 4111 ng/g) when compared with muscle tissue from several species. Further sampling has indicated that toxaphene is elevated in at least one of the large Yukon River lakes upstream of Whitehorse, while concentrations of PCBs and DDTs are substantially lower. Burbot livers from the Yukon River and from several other Yukon lakes contained contaminants at levels lower than or comparable to those found in Mackenzie and Slave River samples (means of <300 ng/g for each of toxaphene, PCBs and DDTs). Possible explanations for the variations in contaminant levels in Yukon River system fish will be discussed.

TRANSPORT OF BLEACHED KRAFT MILL RELATED CHLORINATED CONTAMINANTS IN THE WAPITI-SMOKY RIVER SYSTEM.

C. Ian Johnson, and R. Dean Smillie, Alberta Environmental Centre, Bag 4000, Vegreville, AB, T9C 1T4, (403) 632-8464; and Leigh Noton, Environmental Quality Monitoring Branch, Alberta Environment, 9820 - 106 St., Edmonton, AB, T5K 2J6, (403) 427-5893.

Results of analyses of chlorinated contaminants gathered during synoptic surveys and related monitoring of the Wapiti-Smoky River system are presented and discussed. Downstream of the pulp mill in Grande Prairie, adsorbable organic halide (AOX) is conservative over the system, and pulp and paper related chlorinated phenolic compounds are observed as far as the Peace River confluence. Chlorinated resin acids are only observed in a localized area, immediately downstream of the mill's effluent discharge.

The kinetics of changes in loads of these compounds in the water column, their transformations, and their transportation through the watercourse are discussed.

ZOOBENTHOS AND WATER QUALITY DURING WINTER LOW FLOWS IN A RIVER RECEIVING TREATED BLEACHED KRAFT MILL EFFLUENT AND SEWAGE.

L.R. Noton, Alberta Environment, Edmonton, AB, (403) 427-5893; A.M. Anderson, Alberta Environment, Edmonton, AB; D. Krochak, TAEM, Saskatoon, SK; and L. Steeves, Procter & Gamble, Grande Prairie, AB.

The Wapiti River in NW Alberta receives treated municipal sewage and treated effluent from a 900 t/d bleached kraft pulp mill. Surveys were conducted to assess water quality conditions and effects on zoobenthos during two recent winters; when the BKME comprised 3 to 4% of the river's flow. The taxonomic composition and abundance of benthic macroinvertebrates are described and their response to effluent is assessed. The zoobenthos showed evidence of nutrient enrichment but no clear evidence of toxicity. River concentrations of chlorophenolics, resin acids, ammonia, sulphide, and other pertinent variables are also described.

INTER-LABORATORY COMPARISON OF AN APPROACH FOR SCREENING OF PULP AND PAPER MILL EFFLUENTS WITH RESPECT TO THEIR ABILITY TO CAUSE ELEVATED MIXED FUNCTION OXYGENASE (MFO) ACTIVITY IN FISH.

P.H. Martel*, T.G. Kovacs, B.I. O'Connor, R.H. Voss, Pulp and Paper Research Institute of Canada, 570 St. John's Boulevard, Pointe Claire, PQ, H9R 3J9, (514) 630-4100.

The potential of various secondary-treated pulp and paper mill effluents to induce mixed function oxidase (MFO) activity in the liver of rainbow trout (*Oncorhynchus mykiss*) was investigated by short-term laboratory exposure. A total of twenty four effluents from eight different mills were tested. The study included thermomechanical pulp (TMP), chemi-thermomechanical pulp (CTMP), unbleached kraft and bleached kraft pulp mills. The effects of different kraft bleaching processes on the ability of final mill effluents to cause increased MFO activity were given special consideration.

The results showed that the TMP and CTMP effluents investigated in this study did not increase MFO activity. In contrast, MFO activity was significantly induced as a result of exposure to bleached as well as unbleached kraft mill effluents. The use of chlorine for bleaching did not appear to be a major factor in causing elevated MFO levels. Also, new bleaching technologies and secondary treatment did not eliminate the MFO response in fish. Some preliminary evidence indicated that in fact the kraft cooking process used to convert the wood feedstock into pulp may be a source of MFO-inducing substances.

CHLORINATED ANISOLES AND VERATROLES IN THE ATHABASCA RIVER. IDENTIFICATION, DISTRIBUTION AND OLFACTORY EVALUATION. **B.G. Brownlee and G.A. MacInnis, Environment Canada, Burlington, ON, (416) 336-4706; and Leigh Noton, Environmental Quality Monitoring Branch, Alberta Environment, 9820 - 106 St., Edmonton, AB, T5K 2J6 (403) 427-5893.**

2,4,6-Trichloroanisole (246TCA), 4,5-dichlorovertrole (45DCV), 3,4,5-trichloroveratrole (345TCV) and tetrachloroveratrole (TeCV) have been identified in base/neutral extracts of large volume (20L) water samples collected from the Athabasca River in March, 1991. The presumed source is the effluent from the bleached kraft pulp mill at Hinton. These compounds were detected as far as 1100 km downstream of Hinton. Semi-quantitative analysis gave average concentrations of 1,7,7, and 1 ng/L for 246TCA, 45DCV, 345TCV and TeCV, respectively. The 246TCA concentration is close to the odour threshold concentration, but the concentrations of the chlorinated veratroles close to the odour threshold concentration, are 2 to 4 orders of magnitude below the odour threshold. Odour profile analysis for this series of extracts was carried out by olfactory gas chromatography (nose as detector) and showed several odour peaks in addition to the chlorinated anisole and veratroles. Work on identification of other odour compounds is ongoing.

SURVEY OF ENVIRONMENTAL EFFECTS ASSOCIATED WITH DISCHARGES FROM ONTARIO PULP MILLS.

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ABSTRACT

We examined the receiving areas of 10 Canadian pulp mills, including kraft mills using chlorine, as well as sulphite and TMP mills. Field collections included sampling of receiving water for chemistry and toxicity testing, and sampling of local fish for organ weights, MFO activity, plasma steroid levels, and levels of liver dioxins. Although white sucker collected near bleached kraft mills exhibited the highest MFO induction and levels of dioxins, elevated enzyme activity was observed in fish from sites which did not use chlorine. The absence of chlorine bleaching or the presence of secondary treatment did not eliminate effects on fish, including decreased circulating levels of sex steroids, decreased gonadal size and increased liver size.

Introduction

Studies conducted during 1988 and 1989 at Jackfish Bay, Lake Superior, indicated that white sucker (*Catostomus commersoni*) exposed to bleached kraft pulp mill effluent (BKME) exhibited a wide variety of impacts (McMaster et al., 1991, 1992; Munkittrick et al., 1991; Van Der Kraak et al., 1992). This included delayed sexual maturity, smaller gonads, reduced body size, increased liver size, and elevated mixed function oxygenase (MFO) activity. Plasma levels of sex steroids in these fish were low owing to a reduced secretion of gonadotropin from the pituitary, a decreased steroid biosynthetic capacity by the ovary and testis, and altered peripheral metabolism of steroids (Van Der Kraak et al. 1992). Similar effects on MFO activity, liver size and reproductive fitness were observed in lake whitefish (*Coregonus clupeaformis*) (Munkittrick et al., 1992a) and longnose sucker (*Catostomus catostomus*) (Munkittrick et al., 1992b) at Jackfish Bay. Overall, these effects were consistent with Scandinavian studies of BKME impacts in the Baltic (reviewed in Sodergen, 1989).

The mill discharging into Jackfish Bay installed an aerated stabilization basin, which began operation in September 1989 (Karl, 1992), two years after the initiation of studies on white sucker. To date, there has been no evidence of recovery of reproductive function or alleviation of MFO induction (Munkittrick et al., 1992b), although there has been limited improvement in terms of liver size and condition factor (Munkittrick et al., this issue). Since similar impacts have been identified at another bleached kraft mill with secondary treatment (Servos et al., 1992), we were interested in evaluating whether other Canadian pulp mills exert similar effects.

Methods

We examined the receiving areas of 10 Canadian pulp mills, including kraft mills using chlorine, as well as sulphite and TMP mills which do not use chlorine bleach (Table 1). Each of the mills discharged ~50,000 m^3 day^{-1} of effluent. Fish collections were conducted using overnight gillnet sets within a few km downstream of effluent outfalls (Table 1). Mountain Bay and Black Bay were selected as reference sites for Lake Superior sites, and have been used in previous surveys. Except for the Espanola site where we had previous experience, upstream reference collections turned out to be too time consuming at riverine sites. Two additional reference locations were selected for Northern and Northwestern Ontario sites (Groundhog River and Winnipeg River, respectively).

All samples were collected between the 20th of August and 15 of September, except for Espanola, which was sampled in late October due to a mill shutdown during the September sampling period. All fish were sampled live, and fork length, total weight, liver weight and gonad weight were determined for each fish. Fish were aged using cleaned, dried opercular bones. Samples for MFO activity and plasma steroids were collected and analyzed as described in previous studies (McMaster et al. 1991). Dioxin equivalents were estimated by a modification of the rat H4IIE assay developed by Giesy (van den Heuvel et al., in prep.).

Results and Discussion

Male white sucker were found to have induced levels of hepatic EROD activity, regardless of mill type, although those with chlorine bleaching showed higher elevation (Table 2). Females showed a more variable response, dependent upon receiving environment. Both sexes of fish showed depressions in levels of plasma sex steroids, and although effects were inconsistent at non-chlorinated mills, impacts were observed at some sites without chlorine bleaching (Table 2). Additional changes in liver size and gonadal size were easily detected at some sites without chlorine bleaching. Levels of dioxin equivalents, as measured by the rat H4IIE assay, were non-detectable at reference sites on the Winnipeg River, Groundhog River and Espanola River (Darkie Creek), whereas levels were 3.3 to 8.8 ppt at Black Bay and Mountain Bay reference sites. These levels were similar to those recorded at Red Rock and Kapuskasing, downriver of the pulp mills (Table 2). Levels of dioxins were highest in kraft mills (Table 2), with the exception of Red Rock, which only bleached 5% of its pulp using chlorine at the time of this survey (Table 1). There was a very high correlation between the rat H4IIE estimates of dioxin TEQs and those measured by GCMS (Servos et al., in prep.). There was a significant correlation between dioxin levels in liver and MFO induction in male fish, but no such correlation with any other effects. It is unknown whether dioxins can be causally linked to MFO induction, since a) dioxin levels did not correlate with effects within sites, b) high induction was seen at Red Rock, which had low dioxin levels because it bleached only a small proportion of its pulp, and c) previous experiments have shown that induction in wild, caged and laboratory-exposed fish disappears within 8 d of transfer to clean water, whereas laboratory experiments with dioxins and furans have shown the half-life of induction to be much longer.

Table 1. Mill treatment and receiving water characteristics (based in part on 1990 data from McCubbin et al., 1992).

Mill	Process	Production (ADMT; total including unbleached)	Treatment Primary	Secondary	Outfall	Receiver	River Discharge ($m^3 s^{-1}$)[2]	Dilution	Fish Collection (km downstream)
Marathon	Kraft	425	Clarifiers	none[1]	multiport diffuser	Lake Superior	NA[3]	NA	0.1 - 0.5
Terrace Bay	Kraft	1110	Clarifiers (2)	7-10 d ASB[4]	none	Jackfish Bay, Lake Superior	NA	NA	0.5 from creek mouth
Red Rock	Kraft	57 (819)	Clarifier	none	surface with foam control	Nipigon Bay, Lake Superior	NA	NA	0.5 - 1.0
Thunder Bay	Kraft	1279 (2290)	Clarifiers (4)	none[1]	diffuser	Kaministiquia River, Thunder Bay, Lake Superior	53	26	0.5 - 4.0
Espanola	Kraft	943	Clarifier and settling basins	7-10 d ASB	diffuser	Spanish River, Lake Huron	131	115	2.0 - 5.0
Cornwall	Kraft	412 (726)	Clarifier	none	submerged diffuser	St. Lawrence River	7,340	5,027	5.0 - 7.0
Fort Frances	Kraft	573 (970)	Clarifier and settling basins	3 d ASB	submerged diffuser	Rainy River	277	311	1.0 - 3.0
Kenora	Sulphite-mechanical	929	Clarifier	none	diffuser	Winnipeg River	425	773	2.0
Dryden	Kraft	735 (965)	Clarifier	7 d ASB	underwater	Wabigoon River, Winnipeg River	15	14.2	fish not sampled[5]
Kapuskasing	Sulphite-mechanical	983 (128 low yield sulphite)	Clarifiers	none	-	Kapuskasing River, Moose River, James Bay	78	89	15
Smooth Rock Falls	Kraft	298	Clarifier	none	none	Mattagami River, Moose River, James Bay	113	192	0.5 - 1.5
Iroquois Falls	Sulphite-mechanical	801	Clarifiers (2)	none	diffuser	Abitibi River, Moose River, James Bay	180	249	fish not sampled[6]

[1] under construction; [2] long term (30 year) average flow; [3] Not applicable; [4] Aerated stabilization basin; [5] fish difficult to catch due to summer fish kill; [6] due to time constraints

Table 2. Summary of whole fish responses to pulp mill effluent (data for white sucker only; data for additional sites with low sample sizes or different species not included; + increased, - decreased, 0 no change, * results different from only 1 reference site).

Mill	Process	Effluent Treatment	Males MFO	Males Steroid	Males GSI	Males LSI	Males TCDD TEQs[b]	Females MFO	Females Steroid	Females GSI	Females LSI	Females TCDD TEQs[b]
Terrace Bay	Kraft	Secondary	+	-	-	+	+++	+	-	-	0	+++
Espanola	Kraft	Secondary	+	-	0	0	++	+	-	0	0	++
Fort Frances	Kraft	Secondary	+	-	0	+	++	+	-	-	0	+
Red Rock	Kraft	Primary	+	-	*	*	+	+	*	-	0	+
Smooth Rock Falls	Kraft	Primary	+	-	0	0	+++	+	-	-	0	+++
Kapuskasing	TMP	Primary	+	0	0	+	+	0	*	-	+	+
Kenora	Sulphite	Primary	+	0	-	+	+	0	-	-	+	+
Marathon[a]	Kraft	Primary	+				+++	+				+++
Cornwall[a]	Kraft	Primary	0				not done	0				not done
Thunder Bay[a]	Kraft	Primary	+				++	+				not done

[a] insufficient numbers of fish for additional analysis

[b] liver levels as measured by rat H4IIE assay (+ <10 ppt, ++ <40 ppt, +++ >40 ppt)

Conclusions of this and earlier studies are that a) induction of hepatic MFO enzymes and depressions of plasma sex steroid levels during early gonadal growth are a consistent finding downstream of pulp mills, b) most population level changes evident in wild fish can be correlated with decreased plasma levels of gonadal sex steroids, c) steroid problems are related to a breakdown in the control and production of gonadal sex steroids, and not directly to catalytic activity associated with induced MFO activity, d) secondary treatment does not eliminate these impacts, e) these impacts are seen at some mills without chlorine bleaching, f) dilutions of non-toxic effluent of >200:1 do not appear to remove these effects, g) laboratory toxicity tests on invertebrates, rainbow trout and fathead minnows could not predict impacts on wild fish and h) although the bioassay-derived dioxin equivalents measured using the rat H4IIE assay showed a very high correlation with traditional chemically-derived values at the relatively isolated sites used in this study, there was no relationship of dioxin levels to impacts on steroids, gonad size or liver size.

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PCDD, PCDF, AND EOCl BIOACCUMULATION IN A NORTHERN CANADIAN RIVER SYSTEM.

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Abiotic and biotic compartments in a Northern Canadian river system have been analyzed for polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and extractable organochlorines (EOCl). The water column and both deposited and suspended sediments were analyzed as were invertebrates and several fish species. The organic carbon contents of sediments and the lipid levels of biota were also analyzed for most samples. These initial data suggest that PCDDs, PCDFs, and EOCl may be primarily transported in suspended sediments. The available data support food chain transfer from filter feeding macroinvertebrates to particular fish species such as the mountain whitefish (*Prosopium williamsoni*). Analyses of other benthic-feeding species such as longnose sucker (*Catostomus catostomus)* and piscivorous species such as northern pike (*Esox lacius*) show that bioaccumulation is limited. These data suggest that simple, generalized bioconcentration factors from the water column for compounds such as PCDDs are insufficient. We suggest that regulatory risk assessments should employ site specific models based on the particular characteristics of the individual ecosystem.

BIOLOGICAL RESPONSES OF WHITE SUCKER TO POLYCHLORINATED DIOXINS AND FURANS AT PULP AND PAPER MILLS IN ONTARIO.

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ABSTRACT

Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) were measured in male white sucker liver samples from 7 pulp and paper mill sites, and 3 reference sites in Ontario. The mills selected included 5 bleached kraft mills, with and without secondary treatment, a thermal-mechanical mill and a sulphite-mechanical mill. Biological information including liver weight, gonad weight, mixed function oxygenase activity (EROD) and plasma sex steroids were measured on each individual fish. There was no relationship between TCDD-Toxic Equivalents (TEQ) and liver somatic index, 11-ketotestosterone or gonad somatic index. Although there is a general positive relationship between EROD activity and TEQ, one kraft mill with only minor chlorine bleaching (7%) had low TEQs but elevated EROD activity.

INTRODUCTION

Polychlorinated dioxins and furans have been identified as trace constituents in pulp and paper mill effluents. Considerable effort and expense has gone into measuring the levels of polychlorinated dioxins and furans in fish tissues at pulp and paper mill sites across Canada. Although information exists regarding the potential impact of TCDD/TCDF residues to human consumers our knowledge of the potential impacts on fish populations is limited. The biological significance of TCDD/TCDF residues in fish needs to be addressed in order to enable a realistic assessment of their risk to fish.

Information available from previous studies on wild fish is limited because most of the studies were conducted for predicting only human health impacts. Such studies generally involved measurement of TCDD/TCDF residues in composites of skinless fillets or whole fish collected downstream of only bleached kraft mills. There are seldom any measurements of other biological parameters in these studies and samples sizes are typically small. Differences between studies in methodologies, species, etc., make it difficult to make direct comparisons.

In this study PCDD/PCDF analysis was completed on livers of male white sucker from seven pulp and paper mill sites in Ontario. The sites included mills using kraft (with and without secondary treatment), thermal-mechanical (TMP) and sulphite-mechanical processes (Table 1). Biological data including liver weight, gonad weight, EROD and 11-ketotestosterone were collected on individual fish for which corresponding PCDD/PCDF analysis was conducted.

METHODS

White sucker (*Catostomus commersoni*) were collected at all mill sites (3 at each reference site) using overnight gill net sets between August 20 and September 15, 1991, except in the Spanish River which was sampled in mid October 1991. Collections were part of a larger 10 mill study described by Van Der Kraak et al. (1992). For this study livers of 5-9 male fish were used. Reference sites were Mountain Bay, Lake Superior, Lake of the Woods and the Upper Spanish River. All fish were sampled live and fork length, total weight, liver weight and gonad weight recorded. Samples for MFO and 11-ketotestosterone were taken and analyzed as described in previous studies (Munkittrick et al. 1992).

Congener specific PCDD/PCDF analysis was as described by Huestis and Sergeant (1992). A 3-5 g sample of liver was ground in sodium sulphate and extracted with DCM. Extracts were separated by gel permeation chromatography, cleaned up using micro-alumina and carbon fibre, followed by analysis by High Resolution GC/MS (VG-Autospec). TEQs were calculated using the International Toxicity Equivalency Factors (NATO 1988).

Table 1. Characteristics of the pulp and paper mills where fish samples were collected.

MILL	PROCESS	PRODUCTION (ADMT/D) Total/Bleached		TREATMENT
Terrace Bay	Kraft	1110	1110	Secondary
Espanola	Kraft	943	943	Secondary
Fort Frances	Kraft	970	573	Secondary
Smooth Rock Falls	Kraft	298	298	Primary
Red Rock	Kraft	819	57	Primary
Kenora	Sulphit	929		Primary
Kapuskasing	TMP	928		Primary

RESULTS AND DISCUSSION

2,3,7,8-TCDD and 2,3,7,8-TCDF were the dominant congeners detected at all bleached kraft mill sites with TCDF ranging from 4 to 16 times higher (Figure 1). Only two mills sites, Kenora (sulphite-mechanical) and Red Rock (kraft, 7% bleached) did not have detectable (<0.1-2.7 pg/g) levels of 2,3,7,8-TCDD and levels of other PCDD/PCDF congeners at these sites were within the range of the three reference sites. Mean TEQs in liver samples at the mill sites ranged from 1 to 129 pg/g while mean TEQs at the 3 reference sites ranged from 1 to 3 pg/g (Figure 2).

There was no relationship between liver somatic index, 11-ketotestosterone, or gonad somatic index and TEQs. With the exception of one mill (kraft mill with 7% bleached production) there is a positive relationship between EROD activity and TEQs with an apparent TEQ threshold less than 8 pg/g (Figure 3). Parrott et al. (1992) demonstrated a threshold for induction of 20 pg/g for 2,3,7,8-TCDD and a strong positive relationship between liver dose and EROD activity in rainbow trout given a single oral dose in the laboratory. Although the laboratory results can not be extrapolated directly to the field, because of differences in species, exposure, etc., it is strong supportive evidence that PCDD/PCDFs might be involved.

The three reference sites and the sulphite-mechanical mill all have low EROD activity and low TEQs. The TMP mill has slightly elevated TEQs and EROD while all of the kraft mills have elevated EROD activity. The one apparent outlier casts doubt on the validity of the relationship between PCDD/PCDF and EROD activity. An inducer at this site other than TCDD/PCDD is indicated. Since it is the kraft mills which all have elevated EROD activity, the kraft process may be related to the EROD induction in fish downstream of the mills, and not the treatment, chlorine use or PCDD/PCDF contamination. Further studies at a variety of mill sites will be required to address these questions.

To date most studies have focused on bleached kraft mills and have collected only a limited number of TCDD/TCDF values that correspond to individual fish for which other physiological responses have been determined. Most of the TCDD/TCDF analyses available at these and other sites are for skinless fillets or whole fish which is often analyzed as a composite and the biological data available on individual fish is limited. There is only a limited number of samples for which both biological and chemical data are available. Methodologies (including species) for biological and chemical analysis vary considerably between studies making it difficult to make direct comparisons.

McMaster, M., G. Van Der Kraak, C. Portt, K. Munkittrick, P. Sibley, I. Smith and D. Dixon. Changes in hepatic mixed function oxygenase (MFO) activity, plasma steroid levels and age of maturity of white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. Aquatic Toxicology 21:199-218.

Figure 1. Mean concentration (±s.e.) of 2,3,7,8-substituted polychlorinated dioxins and furans in white sucker liver from the Mattagami River, 15 km down stream of Smooth Rock Falls.

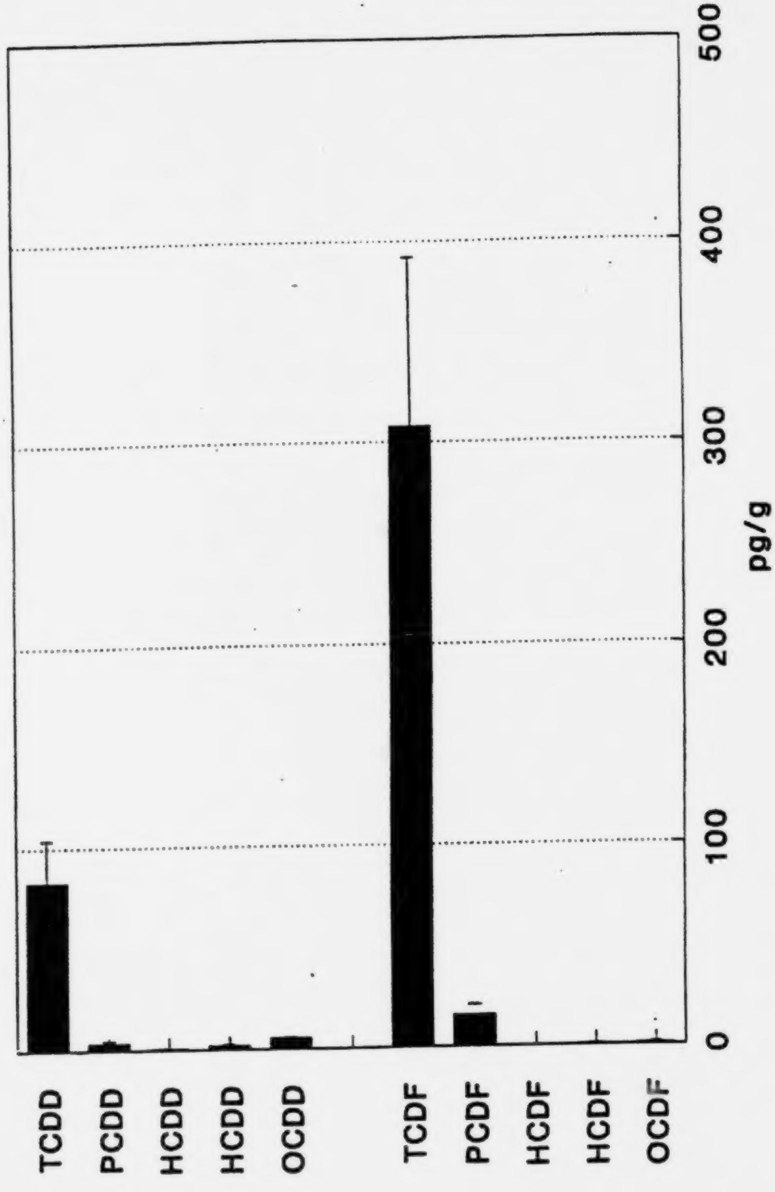


Figure 2. Mean TEQs (±s.e.) in white sucker liver at seven pulp and paper mill sites and three reference sites in Ontario.

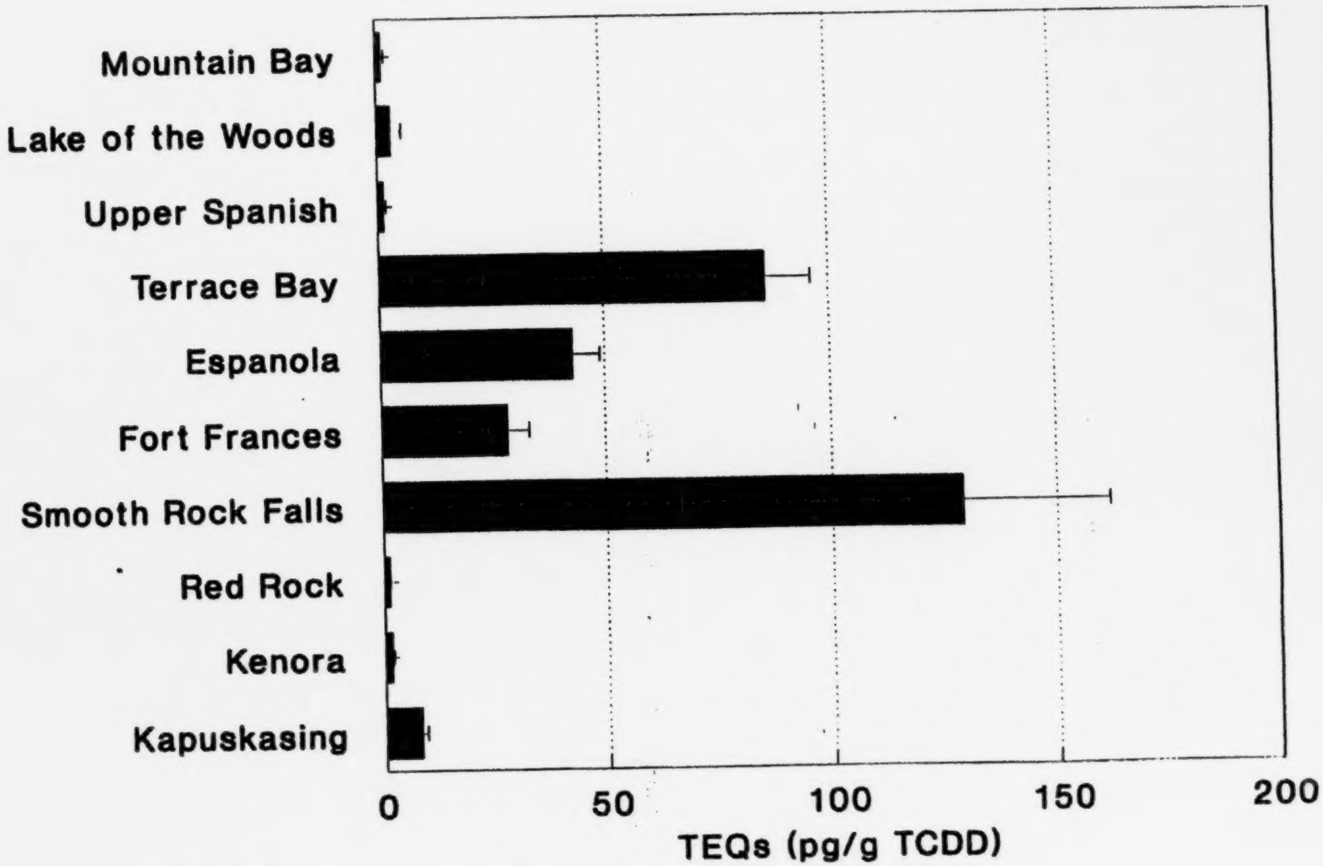
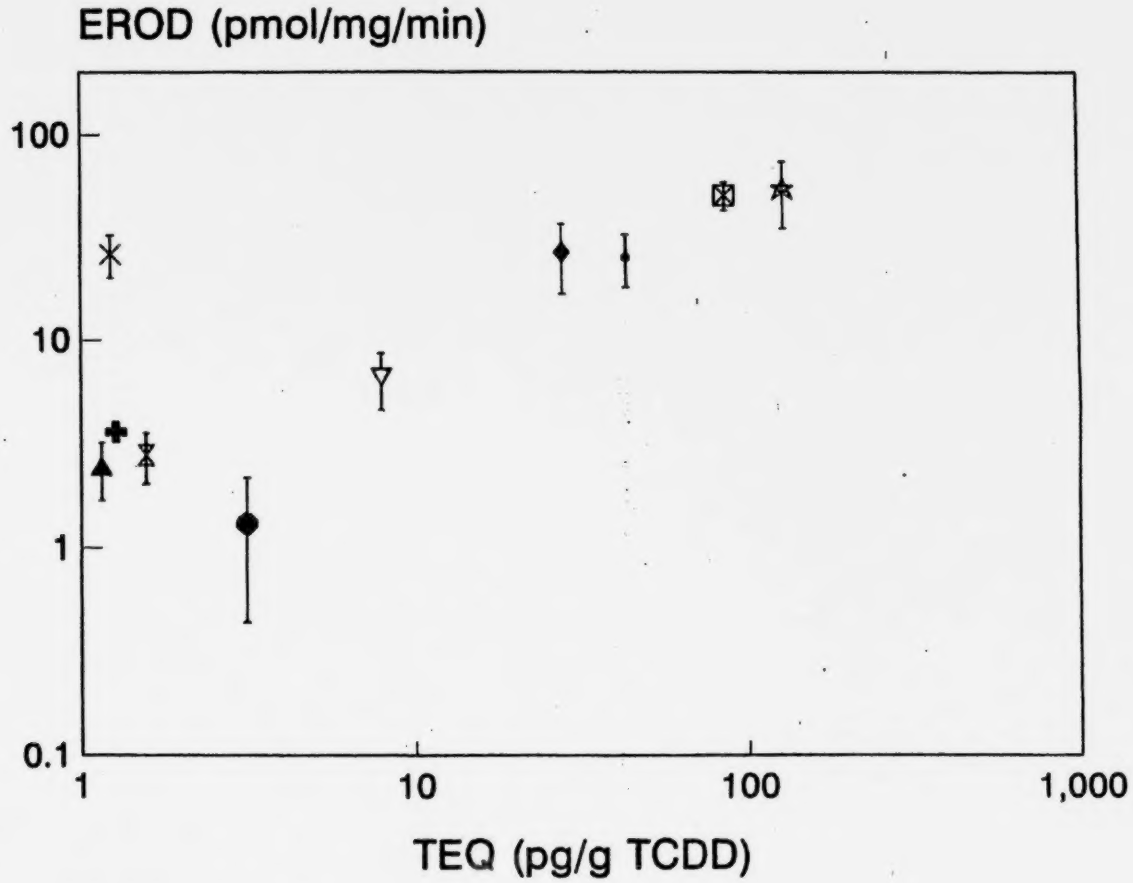


Figure 3. Mean EROD activity relative to mean TEQs in white sucker liver at seven pulp and paper mill sites and three reference sites in Ontario.



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RELEASE OF CHLOROPHENOLICS FROM HIGH MOLECULAR SIZE (>400 D)ORGANIC MATERIAL FROM BLEACHED KRAFT MILL EFFLUENT.

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High molecular size (HMS) organic material (>400 D) isolated from a bleached kraft pulp and paper mill effluent was found to release chlorinated guaiacols and chlorinated vanillins. This phenomena was found to be abiotic and rates of release were temperature dependent. Maximum concentrations of chlorophenolics released were not reached after 32 days of incubation at the highest temperature. Formation of chlorovanillins but not chloroguaiacols was pH dependent with increasing rates at higher pH. Chromic acid and base (2N NaOH) digestions were used to determine the maximum concentrations of chlorophenolics associated with HMS organic material. Low recoveries of these compounds were observed using chromic acid probably due to the oxidative conditions. Base digestion at 100°C gave the highest concentration with maximums of 38, 24, 4.8 and 4.5 μg/L for 4,5-dichloroguaiacol, 3,4,5-trichloroguaiacol, 4,5,6-trichloroguaiacol and tetrachloroguaiacol. Maximums were not attained for 5,6-dichlorovanillin and 6-chlorovanillin even when concentrations reached 49 and 275 μg/L respectively. Organic chlorine released in the form of chlorophenolics accounted for less than 1.5% of the carbon adsorbable organic chlorine (AOX).

A COMPARISON OF BIOASSAY RESULTS FROM UNTREATED CTMP EFFLUENT. Can. J. Fish. Aquat. Sci. E.C. Dombroski, K.L. Smiley, C.I. Johnson, L.Z. Florence, F.P. Dieken. Alberta Environmental Centre, Vegreville, AB, T9C 1T4 and A.A. Qureshi, Norwest Labs, Edmonton, AB, T6E 0P5 and R.N. Coleman, Alberta Environmental Centre, Vegreville, AB, T9C 1T4.

ABSTRACT

Concerns over the impact of industrial effluent on groundwaters and surface waters have led to the use of various biological and chemical assays to evaluate risk and set standards for industrial effluents. The objective of this study was to compare the relative responses of three different acute toxicity bioassays to actual samples of complex toxic effluent. Due to logistical limitations, the study was limited to a single industrial effluent source. Dilutions of synthesized chemical thermo-mechanical pulping (CTMP) effluents were prepared for biological testing using Microtox®, *Daphnia magna*, and rainbow trout (*Oncorhynchus mykiss*), and for comprehensive chemical analysis in order to characterize the organic and inorganic constituents. The results are discussed in relation to how relative responses of the different bioassays compared and how they could impact the evaluation/assessment of effluent toxicity from this particular industrial source.

INTRODUCTION

Alberta Environment has an ongoing industrial monitoring program which involves the participation of the Alberta Environmental Centre (AEC) and the Standards and Approvals Division of Environmental Protection Services. The objective of this research project is to assess the environmental impact of various industrial effluents by using a combination of biological and chemical analyses. In monitoring the toxicological impact of industrial effluents it is desirable that tests which must be carried out routinely meet certain criteria. These include using generally accepted or standardized tests which are cost effective, rapid, relatively simple, have minimal logistical and labour requirements and are sensitive to the toxicants in the particular effluent being tested. With respect to bioassays, a single species biological test is usually considered inappropriate to ascertain the potential toxic impact of effluent on all resident biota in receiving waters. Therefore, a battery of single species or multi species tests may be desirable. Chronic tests are often more sensitive than acute tests, however, they are generally much more time-consuming, complicated and therefore costly. An increase in variety of analyses also comes with an associated increase in cost. It would be advantageous to include in a battery of tests, some that are relatively quick, simple and cost-effective. These rapid tests would be useful in the early detection of potentially toxic effluents, or process streams and in preliminary range-finding to ensure appropriate dilutions are used in more labour intensive, time-consuming and costly bioassays.

Three of the bioassays commonly used in the industrial effluent monitoring program are the acute 48 h static *Daphnia magna* test, the acute 96 h static rainbow trout (*Oncorhyncus mykiss*) test, and the Microtox® test, which represent three different trophic levels. The first two tests are fairly widely accepted by regulatory agencies with associated costs ranging from $300 to $2000 or more per test. The latter test is gaining popularity as a short-term assay (taking only minutes to perform) which is available at reasonable cost (about $150). Some comparisons of the relative responses of these bioassays have been made by researchers investigating the toxicity of complex effluents, contaminated waters and wastewaters, and sediments (Munkittrick et al. 1991, Firth and Backman 1990). However, data on CTMP effluent is minimal and many studies do not include well-coordinated subsampling for simultaneous biological and chemical analysis.

Effluents of three CTMP mills included in the preliminary work of this study did not elicit a toxic response from any of the three traditional bioassays being conducted. For this reason, sampling was limited to a single effluent source into which raw untreated effluent (extremely toxic) was added to obtain a sample of synthetic toxic effluent blend which was suitable to the purpose of this study.

In this paper we present and discuss the results to date, comparing the relative toxic responses of these bioassays to spiked CTMP mill effluent. We also present some preliminary data on the comprehensive chemical analysis of spiked CTMP mill effluent which, along with data from future studies, will be used to relate chemical composition to the toxic response observed in the bioassays.

METHODS

The Microtox® Assay Procedure described in Part 3, 2.0 of the AEC Microbiological Methods Manual (Qureshi 1990) was used for the determination of Microtox® toxicity. It is a short-term acute toxicity assay in which dilutions of samples were added to suspensions of luminescent bacteria (*Photobacterium phosphoreum*) and incubated for 15 minutes at 15°C. Luminescence was measured on a Microtox® Model 2055 instrument (Microbics Corporation, Carlsbad, CA) and results were reported as the sample concentration (%) at which a 50 per cent decrease in luminescence is observed after 15 minutes (15 minute EC_{50}). Due to the marginal effect of sample colour on observed luminescence, the values reported here were not corrected for colour.

The 48 h static acute toxicity test using *D. magna* followed the protocol outlined in the AEC Standard Operating Procedures of the Aquatic Biology Branch (1991). Results were reported in terms of an LC_{50}, the sample concentration (%) that was lethal to 50% of the test organisms over the duration of the test.

The 96 h static acute toxicity bioassay using rainbow trout followed the protocol outlined in the AEC Standard Operating Procedures of the Aquatic Biology Branch (1991). Results were reported in terms of an LC_{50}, the sample concentration (%) that was lethal to 50% of the test fish over the duration of the test.

Following collection of the raw and final effluent, subsamples of each were then taken for Microtox® and chemical analysis. Based upon the Microtox® results, and our previous experience with this effluent, 80 L volumes of blended raw and final effluent were prepared (in the general range of 10-30% untreated effluent and 70-90% treated effluent) in order to obtain an effluent blend with a toxicity range suitable to allow comparison of the relative sensitivities of the bioassays (ie., below about 10% untreated effluent, no fish mortalities would be expected and above 30% untreated effluent 100% mortality would be expected). Due to logistical limitations, a maximum of three sample blends could be tested concurrently. Sample blends were then aerated at 60 mL min^{-1} L^{-1} for 10 days at room temperature (this was necessary in order to reduce the biological oxygen demand (BOD) of the effluent blends, otherwise gentle ($\leq$ 7.5 mL $min^{-1}L^{-1}$) aeration during the trout assay would fail to maintain oxygen at levels sufficient to maintain the test fish and the effects of any toxicants would be masked). Dissolved oxygen levels, temperature, pH, and conductivity were recorded daily. After 10 days of aeration, each effluent blend was subsampled for Microtox®, rainbow trout, *D. magna*, organic chemical and inorganic chemical analyses. Following completion of the rainbow trout assay, the lowest serial dilutions of sample blends which showed lethal effect on fish were also subsampled for Microtox® and inorganic chemical analysis, the intent being to help elucidate threshold concentrations of toxicants (with respect to rainbow trout lethality).

Organic chemical analysis included testing for at least 34 volatile priority pollutants, 56 extractable priority pollutants, resin acids and fatty acids using Method Nos. A102.1, Automated Analysis of Volatile Organics in Surface Water; A105.1, Extractable Priority Pollutants in Surface Water; and AE129.C, Resin and Fatty Acids in Pulp Mill Effluents and Receiving Waters of the AEC Methods Manual for the Chemical Analysis of Trace Organics and Pesticides in Environmental Samples (Smillie 1992).

Inorganic chemical analysis included testing for the parameters which are listed, with their corresponding NAQUADAT Method No., in Table 1, and were carried out as described in the Methods Manual for Chemical Analysis of Water and Wastes (Dieken 1987).

Table 1. Tests conducted on CTMP effluent for inorganic chemical parameters.

Parameter	NAQUADAT No.	Parameter	NAQUADAT No.
pH	10301L	Colour (True Hazen Units)	02022L
Calcium	20107L	Cyanide	06606L
Magnesium	12105L	Phenols (4-AAP)	06537L
Total Hardness	10602L	Sulphide	16200L
Sodium	11103L	Cadmium	48009L
Potassium	19103L	Copper	29009L
Conductivity	02041L	Nickel	28009L
Total Dissolved Solids (Calculated)	00205L	Cobalt	27009L
Silica	14107L	Zinc	30009L
Chloride	17206L	Manganese	25003L
Sulfate	16306L	Chromium	24009L
Fluoride	09107L	Vanadium	23009L
Total Alkalinity	10101L	Molybdenum	42009L
Bicarbonate	06201L	Lead	82302L
Nitrate & Nitrite Nitrogen	07105L	Arsenic	33011L
Nitrite Nitrogen	07205L	Selenium	34011L
Total Kjeldahl Nitrogen	07021L	Aluminum	13030L
Dissolved Ammonia Nitrogen	07562L	Beryllium	04103L
Total Phosphorous	15421L	Iron (ICP)	26009L
Sum of Cations	00120E	Barium	56009L
Sum of Anions	00125E		
Chemical Oxygen Demand	08304L		
Biological Oxygen Demand	08202L		

RESULTS AND DISCUSSION

Relative Response of Bioassays.

Rainbow trout, Microtox® and *D. magna* assays were carried out on samples of blended CTMP effluent that were collected from June to August of 1992. Figure 1 shows a comparison of the relative toxicity responses to several of these effluent blends.

The Microtox® assay gave a toxic response in six of the nine cases shown. With a single exception, each of those cases also gave a toxic response in the rainbow trout assay. In the exceptional case the toxic response of the Microtox® assay was marginal, having a 15 min EC_{50} of 95.6%. The Microtox® assay compared favourably with the rainbow trout assay and, in general, appeared to be more sensitive (showed a toxic response at a lower concentration).

The *D. magna* acute assay was much less sensitive than the other two, in fact, no mortalities were observed - even in the intensely aerated sample blends which contained 30% raw effluent. These results tend to agree with those of similar studies carried out on complex industrial (Munkittrick et al. 1991) and kraft mill effluents (Qureshi et al. 1980, Renberg, 1992) which compare the Microtox® and rainbow trout assays. Further statistical correlation of rainbow trout LC_{50} and Microtox® will be conducted as our database increases.

Based on these experimental results, Microtox® would be a useful tool for screening this type of effluents prior to conducting more costly, time consuming and labour intensive rainbow trout assays. This would aid in identifying samples likely to elicit toxic responses in, and for preparing serial dilutions of sample in a range appropriate for the rainbow trout assay. With respect to the *D. magna* acute 48 h assay, our results using these intensively aerated samples of spiked CTMP mill effluent indicate that this was an inappropriate test for this particular type of effluent. Under these circumstances, it may be desirable to look at a chronic assay, such as the seven-day *Ceriodaphnia* chronic bioassay, which has been shown to have a similar and even more sensitive response to kraft mill and industrial effluent than does Microtox® (Firth and Backman 1990, Jop 1992, Renberg 1992), and which represents a similar trophic level in the food chain.

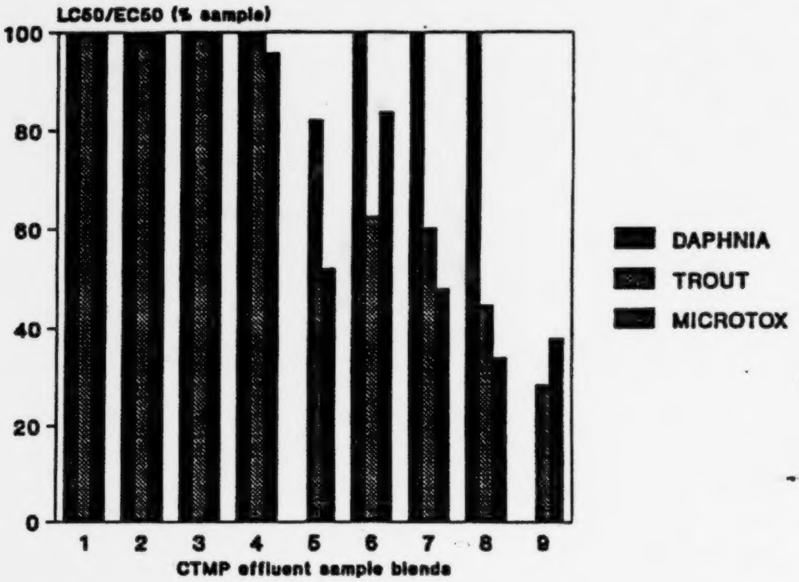

Figure 1. Bioassay responses to blends of CTMP effluent from a single source (in order of increasing toxicity to trout).

The high BOD of this type of synthetic toxic effluent made it impossible to maintain oxygen levels high enough to carry out the trout tests. Therefore, the sample was modified by carrying out a 10-day aeration prior to conducting the comparative assays. Decreasing toxicity of subsamples withdrawn for Microtox® analysis during the course of aeration confirmed that this process adulterated the sample blend, ie, there is a decrease in toxicity over time due to chemical activity, biological activity and stripping of volatiles. (Microtox® could therefore be useful in monitoring the decrease in toxicity along the waste treatment process stream). This treatment was considered to be acceptable since the residual toxicity in the sample was sufficient to allow a comparison of the relative responses of the bioassays. With respect to the rainbow trout assay, this test would be inappropriate for effluent which characteristically has a BOD high enough to cause mortality in trout (ie. this would mask the effect of any toxicants present, and a chemical assay would suffice to monitor BOD alone). However, in this type of effluent, toxicity was still observed in the trout assay following aeration sufficient to compensate for the BOD. This indicates that the trout assay would still serve to detect residual toxicants at the end of the waste treatment process stream.

Table 2. Upper ranges of potential toxicants detected in raw CTMP effluent.

Compound(s)	Levels Detected (mg/L)
Zn^{2+}	0.5 - 1
Mn^{2+}	1 - 2
Phenols (4-AAP)	1 - 2
Total Resin Acids	30 - 40
Total Fatty Acids	15 - 20
Total Organics Detected	10-20

Chemical Characterization of CTMP Effluent

Table 2 lists a number of groups of compounds which have been detected in the untreated CTMP effluent and which may contribute significantly to the toxicity observed in the bioassays. Major components included under total resin acids are primaric, isoprimaric, palustric, abietic, neoabietic, and dihydroabietic acids. Total fatty acids included myristic, palmitic, stearic, oleic, linoleic and arachidic acids. Benzoic and hexadecanoic acids were major components of the aromatic and aliphatic acids which were usually detected. Mono- and - diterpenoids were also usually present (up to 2 mg/L). All of the individual compounds named were found in highly variable concentrations between samples during the four-month period that sampling was conducted. Many of these variations could be attributed to variations in mill operation (eg. substituting birch for aspen as a starting material) or maintenance procedures (eg. shutdown/start-up conditions, flushing of aeration lagoons, etc.).

Due to the variability in the composition of the untreated effluent, more data is required to relate concentrations of specific toxicants or groups of toxicants to observed biological effects. Also, during the 10 day aeration period used to decrease BOD, there was a large increase in microbial biomass. This biomass occurred in the form of a floc which was loosely attached to the surface of the plastic barrel liners, or floating freely in the sample. The amount of growth increased with increasing concentrations of raw effluent. Problems in evenly distributing this biomass during subsampling and preparing serial dilutions for the bioassays were encountered. This greatly confused the relationship between toxicant concentration and observed toxic effect. Sample blends containing greater biomass seemed to possess a lower than expected toxicity. This may have been due to metabolism of toxicants by the microbial biomass and/or to adsorption of toxicants on the organism's greatly increased surface area thus reducing the bioavailability of the toxicants to the bioassay organisms.

The observed toxicity in the trout assays, could not be attributed to pH, which was between 8.0 and 10.0 or to ammonia levels which were less than 1 mg/L. The levels of Zn^{2+} (< 1 mg/mL) and Mn^{2+} (< 2 mg/mL) were less than those usually associated with toxic effects, however they were sufficiently high that they could be contributing to the overall toxic response through synergistic effects. From our experience using their sulfate salts as reference toxicants, concentrations which would elicit a 15 minute EC_{50} in the Microtox test are approximately 1.7 mg/L for Zn^{2+} and 6.0 mg/L for Mn^{2+}.

Firth and Backman (1990) found that, in kraft mill effluent, resin acids and total BOD values were the best predictors of Microtox® response. They did not observe a significant correlation with total phenolics. During our study, BOD effects were controlled through intense aeration. Based on our results to date, levels of resin acids and 4-AAP phenols indicate that these parameters and possibly zinc and manganese should receive further attention.

Using the methods described, no single toxicant was detected in amounts that could unequivocally account for the toxicity observed in the bioassays. Barring the under estimation of certain toxicants, or the presence of some other undetected toxicant, it is likely that interactions between sub-lethal levels of toxicants are responsible for the observed toxicity (ie, additive or synergistic effects). Further investigation is required in order to elucidate the relationship between toxicants and toxic effect for this complex effluent.

CONCLUSION

Samples of blended raw and final effluent from a single CTMP mill were tested concurrently with the Microtox® (15 minute), rainbow trout acute 96 h static and *D. magna* acute 48 h static bioassays. Results indicated that, based on their relative toxicity responses to this particular effluent, the Microtox® assay had potential for use as a rapid and economical screen for samples prior to testing by the rainbow trout assay. Microtox® was also useful for predicting an appropriate dilution series for use in the rainbow trout assay, thus minimizing the numbers of test fish required. Additional data are required for a more complete description of the relationship between the responses of these two assays. The *D. magna* acute assay had a significantly lower sensitivity when compared to the Microtox® or rainbow trout assays, in fact, no mortalities were observed even in sample blends which contained 30% untreated effluent. Therefore, this test does not appear to be as useful for detecting the presence of toxicants likely to occur in this type of effluent. An alternative test such as the *Ceriodaphnia* seven-day chronic bioassay may prove to be more suitable and represent the same trophic level. Further investigations are necessary in order to confirm this.

Chemical analyses have detected the presence of zinc, manganese, phenolics, mono- and diterpenoids, benzoic acid, hexadanoic acid, resin acids, fatty acids, and a variety of other unresolved aromatic and aliphatic hydrocarbons and acids. The accumulation of additional data will be required in order to clarify which components contribute to the toxic responses observed in the bioassays.

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POPULATION RESPONSES IN FISH EXPOSED TO BLEACHED KRAFT MILL EFFLUENT (BKME). S. Swanson, R. Shelast, and R. Schryer, SENTAR Consultants, 200 - 1122 - 4th St., S.W., Calgary, AB, T2R 1M1., K. Kroeker, North South Consultants, Winnipeg, MN, R3T IY7.

ABSTRACT

The potential impact of bleached kraft mill effluent (BKME) discharges on fish populations was studied in the Wapiti/Smoky River system in northwestern Alberta. The field program included seasonal chemical and biological sampling for a wide range of parameters. Fisheries analyses began with species abundance and distribution and for two target species, longnose sucker (*Catostomus catostomus*) and mountain whitefish (*Prosopium williamsoni*), included growth, recruitment, age distribution, mortality and fecundity. Fish movement throughout the study area was determined through radiotelemetry supplemented by anchor tag and recapture techniques. Fish movement studies showed that some fish used the habitat immediately downstream of the mill for overwintering and/or as a summer feeding area. However, fish movement could be extensive and rapid, both during spawning and at other times of the year. Therefore, direct measurements of chemical exposure were required to confirm the degree of exposure to mill effluent. There were no significant differences between exposed and reference fish in terms of length-weight regressions; growth; gonad somatic indices; fecundity; and, mean age of mature fish. Longnose suckers from the Wapiti/Smoky had higher condition factors than reference fish. There were higher numbers of older fish in the reference system. Natural phenomena, particularly a 1:100 year flood in 1990, were important determinants of species distribution, relative abundance and recruitment.

INTRODUCTION

Significant ecological impacts have been documented in waters receiving BKME from mills with little or no treatment and/or low dilution in Scandinavia and Canada (Kautsky et al. 1989, Neuman and Karas 1988, Sandstrom et al. 1988, Anderson et al. 1989, Farara et al. 1988, Munkittrick et al. 1991, Hodson et al. 1991). There are fewer documented impacts in waters receiving BKME from modern mills (with secondary treatment and updated processes such as chlorine dioxide substitution or oxygen delignification). Studies at Monsteras, Sweden, (with secondary treatment and high dilution) show no current impacts on the benthos, fish populations or fish physiology/biochemistry despite previous ecosystem disruptions from chlorate discharges (Grahn 1991). Some impacts were still observed in the Spanish River, Ontario downstream of a mill utilizing oxygen delignification, chlorine dioxide substitution and secondary treatment; however, these were reduced to induction of hepatic mixed function oxidase activity, increased liver somatic indices and increased plasma glucose levels (Servos et al. 1991). Furthermore, dilution at this site can be very limited (up to 10% effluent).

This study was conducted in the Wapiti/Smoky river system which receives biologically treated BKME from the Procter and Gamble Cellulose pulp mill at Grande Prairie, Alberta. Regular benthic monitoring dating from pre-operational baseline work indicates that organics and nutrients from an upstream municipal wastewater treatment plant and from the mill have resulted in moderate enrichment, but "pollution sensitive" species remain (TAEM 1989). The overall goal of this study was to provide data on abiotic and biotic compartments of the river system, with respect to both fate and effects. Various levels of biological organization were examined, from individual physiological parameters to population and community-level indices. This paper reports on the results of the fish population component of the study.

MILL AND STUDY AREA

The mill began operation in 1974 with a six stage bleach plant using molecular chlorine in the first stage followed by alkaline extraction, hypochlorite, chlorine dioxide, alkaline extraction and chlorine dioxide (CEHDED). The effluents were treated in aerated lagoons prior to discharge since start-up. In 1989, the chlorine charge in the first stage was reduced and chlorine substitution increased to 25%. In the fall of 1990, chlorine dioxide substitution was raised to 70%, oxygen reinforcement of the first extraction stage was begun and the hypochlorite stage was eliminated. These steps yielded an effluent without detectable 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and 2,3,7,8 tetrachlorodibenzofuran (TCDF) by the spring of 1991 (Joshi 1991). In July of 1991, 100% chlorine substitution was implemented.

The study area was a 300 km portion of the Wapiti/Smoky river system in northwestern Alberta, from 70 km upstream to 230 km downstream of the mill. The reference system was the upper North Saskatchewan River upstream of Rocky Mountain House, Alberta, which has a similar watershed and habitat characteristics but no BKME discharge. Sampling was conducted in the summer and fall of 1990, and the spring, early summer and fall of 1991. During this time, flows ranged from a 1:100 year flood in July 1990 (4200 m^3/s) to low fall flows of 16 m^3/s.

Major habitat characteristics in the upstream portions of the study area were: (1) riffles usually 20-100% of flow; (2) channel frequently confined; (3) primarily cobble/rubble substrate, sometimes with silt; (4) no significant channel cover; (5) low to moderate stream gradient; (6) repose to steep banks varying from falling to stable; (7) occasional algae cover on substrate; and (8) low invertebrate density.

Major habitat characteristics of the downstream portion of the study area were: (1) primarily run with some riffles and deep pools; (2) channel occasionally confined; (3) cobble/pebble/gravel substrates always covered with silt; (4) no significant channel cover; (5) low stream gradient; (6) flat to steep banks with high degree of instability; (7) frequent heavy algae cover on substrate; and (8) high invertebrate density immediately below the mill and low density farther downstream in the Smoky River. Habitat characteristics in the upper North Saskatchewan were similar to the upstream Wapiti sites, although run habitat was more predominant.

METHODS AND MATERIALS

General fish abundance, distribution and population data were collected for all fish species captured during collections. Two fish species were selected for intensive study; the longnose sucker (*Catostomus catostomus*) and the mountain whitefish (*Prosopium williamsoni*).

Fish were collected primarily by electroshocking, supplemented by gill netting or angling. Each fish caught was weighed and measured, had aging materials taken and any external pathology noted. If the fish was large enough, a plastic anchor tag was attached in order to obtain migration data; a small number of large mountain whitefish and longnose suckers were radio-tagged for direct tracking of movements.

Fish aging was conducted according to the methods of Mackay et al. (1990). Length-weight relationships were determined by regression analysis. Standard growth curves (mean length vs. age class) and Walford-Ford plots for growth coefficients were produced for both target species. Mortality was estimated via a regression analysis of catch frequency versus age plots. Fecundity was determined by counts of 1 gram sub-samples of ovaries. Data for exposed and reference fish were compared by ANOVA for fecundity, age of maturity, gonad somatic indices and condition factors and by ANOVA for length-weight regressions.

RESULTS AND DISCUSSION

Fish species diversity and abundance differed with site, season and year, with greater species diversity in the Wapiti/Smoky system (Table 1). The greater species diversity in the Wapiti/Smoky was due to a combination of the greater variety of habitats and greater sampling effort in that system compared to the reference site.

Seasonal distribution and abundance variations were related, in part, to river flow variations and subsequent effects on fish habitat preference and sampling efficiency. Extreme flow events had profound effects. In June, 1990, Wapiti River flows over 4000 m^3/s appeared to flush most species out of the study area, resulting in catch-per-unit-effort (CPUE) estimates of 12-36 fish per hour of shocking. Low October, 1990 flows of 16-26 m^3/s led fish to congregate in widely separated areas of deeper water. Highest catches during this period were immediately downstream of the mill discharge, where the CPUE was 139 per hour. During more normal flows in 1991, relative abundance recovered, with CPUE's of 195-261 per hour for downstream sites.

Radiotelemetry and anchor tag data confirmed that mountain whitefish and longnose sucker can be very mobile in response to flow, temperature and life cycle events such as spawning. Although spawning movements were often the most pronounced, significant movements were noted for certain individuals at other times of the year. Radio-tracked fish covered distances exceeding 200 km, sometimes over a very short period of time (2-3 weeks) (Figure 1). There was evidence that some fish remained in the "near-field" area downstream of the discharge for over-wintering and for summer feeding. However, because not all individuals remained "resident", concurrent contaminant and biomarker data was required to confirm exposure.

Fish population parameters did not display the suite of characteristics associated with exposure to BKME in some other studies; ie. slower growth, lower reproductive commitment, higher condition factors and delayed maturation (Munkittrick et al. 1991). Longnose suckers from the Wapiti/Smoky did have higher condition factors (Table 2); however, this was likely due to a combination of the larger food base in the Wapiti/Smoky (because of nutrient enrichment downstream) and less ideal habitat in the upper North Saskatchewan (as reflected in species distribution). Longnose sucker growth curves were similar although the lack of younger age classes from the North Saskatchewan site hampers comparison (Figure 2). Mountain whitefish, with higher body burdens of contaminants, had similar condition factors and growth in the Wapiti/Smoky and the North Saskatchewan (Table 2, Figure 3).

Age frequency distributions were different in the Wapiti/Smoky compared to the reference site. There were higher numbers of old fish in the North Saskatchewan and higher numbers of 4-6 year-old mountain whitefish and 6-11 year-old longnose suckers in the Wapiti/Smoky (Figure 4). An extreme flow event in June of 1972 (3680 m^3/s) may help explain the absence of old fish in the Wapiti; this flood may have caused the loss of a year class.

Mortality estimates for longnose suckers and mountain whitefish were significantly lower for the North Saskatchewan than for the Wapiti (Table 3). The higher mortality estimates in the Wapiti reflect the absence of old fish, as illustrated by the age frequencies. The flood in 1972 may be one factor. Exposure to mill effluent may be another factor. However, it was expected that other indicators of health would also be affected if mill effluent caused fish to die at an earlier age; these indicators were not significantly different from reference fish. The very small number of old fish caught at both sites caused difficulties with the analysis; a very small shift in sample number created large differences in mortality estimates. Further sampling would be required for greater confidence in mortality estimates.

Reproductive commitment was similar among exposed and reference populations. Gonad somatic indices and mean age of mature fish were not significantly different (Table 4). Development of secondary sex characteristics occurred normally in the exposed populations. Fecundity was not significantly different in longnose suckers. Unfortunately, mountain whitefish sample sizes for fecundity were too low to permit comparisons in 1990, while 1991 sample collection failed due to a very sudden freeze-up on both the Wapiti and the North Saskatchewan. Recruitment was affected by the 1990 flood; very few young-of-the-year of any species were collected in the Wapiti/Smoky. By 1991, recovery of recruitment appeared to be taking place. Numbers of young mountain whitefish captured in beach seines or observed during electroshocking were higher in the Wapiti/Smoky in 1991 than in the reference river. By 1992, recruitment appeared to have completely recovered. Large numbers of young longnose suckers and mountain whitefish were found immediately downstream of the mill discharge.

Data from the Wapiti River for the period before mill operations began allowed comparisons with fish collected during this study. Length-weight regressions for both longnose suckers captured in 1971 and walleye captured in 1970 and 1971 were not significantly different from regressions for these two species from 1990/91. Condition factors in 1970/71 fish were lower. Thus, overall growth was similar but 1990/91 fish were "fatter", probably as a result of nutrient enrichment downstream of the Grande Prairie sewage outfall and the mill discharge.

Table 1. Species Composition (%) in the Wapiti and North Saskatchewan Rivers.

	Reference				Wapiti/Smoky			
	1990		1991		1990		1991	
	%	n	%	n	%	n	%	n
Brown Trout	0	0	0.2	1	0	0	0	0
Burbot	0	0	2.4	10	2.7	16	9	78
Bull Trout	0	0	0	0	1.9	11	0.5	4
Flathead Chub	0	0	0	0	12.4	72	18.2	157
Goldeye	0	0	0	0	1.5	9	6.4	55
Artic Grayling	0	0	0	0	2.2	13	0	0
Longnose Sucker	58.3	14	23.9	101	32.0	186	26.6	230
Largescale Sucker	0	0	0	0	7.2	42	4.3	37
Mountain Whitefish	41.7	10	72.3	305	19.6	114	8.3	72
Northern Squawfish	0	0	0	0	0.2	1	0.1	1
Northern Pike	0	0	0	0	1.4	8	2.4	21
Slimy Sculpin	0	0	0	0	0	0	0.1	1
Trout	0	0	0	0	0.2	1	0	0
Walleye	0	0	1.2	5	7.7	45	4.4	38
White Sucker	0	0	0	0	11	64	19.8	171
Total	100	24	100	422	100	582	100	865

Table 2. Length - Weight and Condition Indices for the Two Major Fish Species: Wapiti/Smoky Versus the Reference River (North Saskatchewan), 1991.

Species	Length-Weight Relationship				Condition Factor		
	n	Slope (b)	95% C.L. of b	r^2	n	Mean	Standard Deviation
Longnose Sucker							
Wapiti	255	3.03	0.11	0.98	255	1.23	0.17
Smoky	42	2.73	0.19	0.97	42	1.21	0.17
Reference	85	2.78	0.11	0.99	85	1.10	0.13
Mountain Whitefish							
Wapiti	83	2.89	0.16	0.96	83	1.20	0.18
Smoky	-[1]	-	-	-	11	1.07	0.17
Reference	266	2.92	0.08	0.97	266	1.14	0.22

1. Insufficient numbers to derive length - weight relationship.

Table 3. **Mortality Estimates for Longnose Suckers and Mountain Whitefish in the North Saskatchewan and Wapiti Rivers.**

Species	River	Season	Ages Used	In To
LNSK	Wapiti	Combined '90	6-11	
	Smoky	Combined '90	6-11	
	Wapiti/Smoky	Combined '90	6-11	
	Wapiti	Spring '91	8-12	
	Smoky	Spring '91	9-12	
	Wapiti/Smoky	Spring '91	10-16	
	N. Saskatchewan	Spring '91	10-16	
MTWT	Wapiti	Combined '90	6-10	
	Wapiti/Smoky	Combined '90	6-10	
	Wapiti	Spring '91	6-10	
	Wapiti/Smoky	Spring '91	8-12	
	N. Saskatchewan	Spring '91	7-13	
	Wapiti	Combined '91	6-10	
	Wapiti/Smoky	Combined '91	8-12	
	N. Saskatchewan	Combined '91	7-13	

Note: Mortality estimates with different letters are significantly different.

Table 4. Reproductive Indices for the Two Major Fish Species, 1990-1991.

Species	Total Fecundity			Gonad Somatic Index						Mean Age of Mature Fish 1990 - 1991 Combined						Egg Diameters
	n	mean	Standard Deviation	n 90	n 91	mean 90	mean 91	Standard Deviation 90	Standard Deviation 91	n Male	n Female	mean Male	mean Female	Standard Deviation Male	Standard Deviation Female	(1991 Only)
Longnose Sucker																
Wapiti	12	21,187	3,434	12	85	0.03	0.04	0.02	0.05	28	37	7.8	8.5	2.1	2.6	2.128 ± 0.224
Smoky	-	-	-	11	20	0.03	0.04	0.02	0.04	11	15	7.8	8.8	1.7	2.1	
N. Saskatchewan	5	23,124	6,307	10	24	0.04	0.02	0.02	0.02	10	15	9.5	10.8	3.2	2.8	2.358 ± 0.462
Mountain Whitefish																
Wapiti		(2)		22	33	0.06	0.006 [1]	0.08	0.005	8	6	6.4	6.5	1.8	1.9	
Smoky				3	6	0.003	0.006 [1]	0.001	0.005	1	8	5.0	7.8	0.0	4.1	
N. Saskatchewan		(2)		10	31	0.03	0.007	0.02	0.004	8	21	7.4	8.0	1.6	2.8	

1. Spawning period data for 1991 not available.
2. Insufficient sample size.

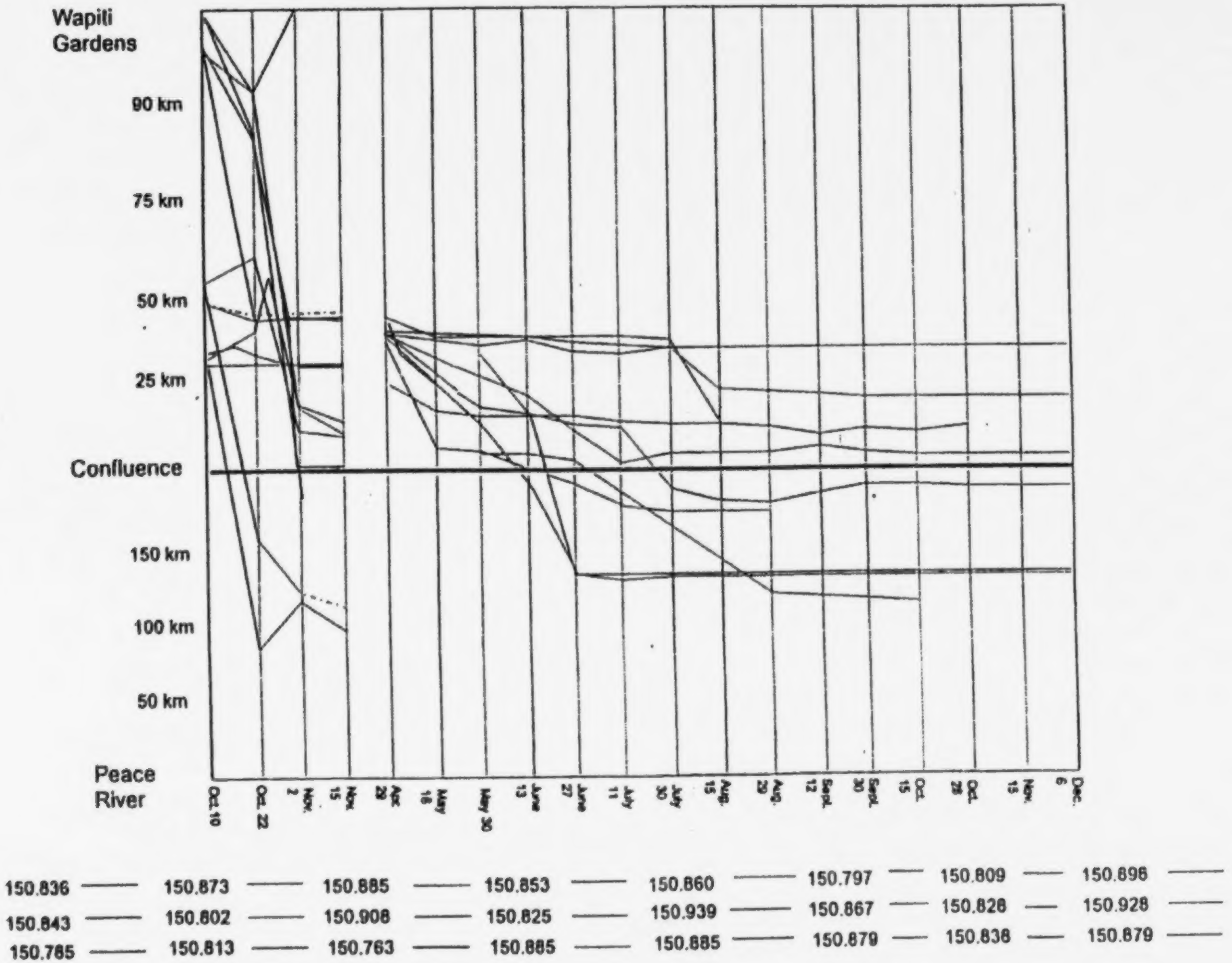


Figure 1. Summary of Fish (Mountain Whitefish) Movement as Tracked by Radiotelemetry, 1990 and 1991.

Figure 2. Growth Curves for Longnose Suckers from the Wapiti/Smoky and North Saskatchewan River Systems - Spring, 1991.

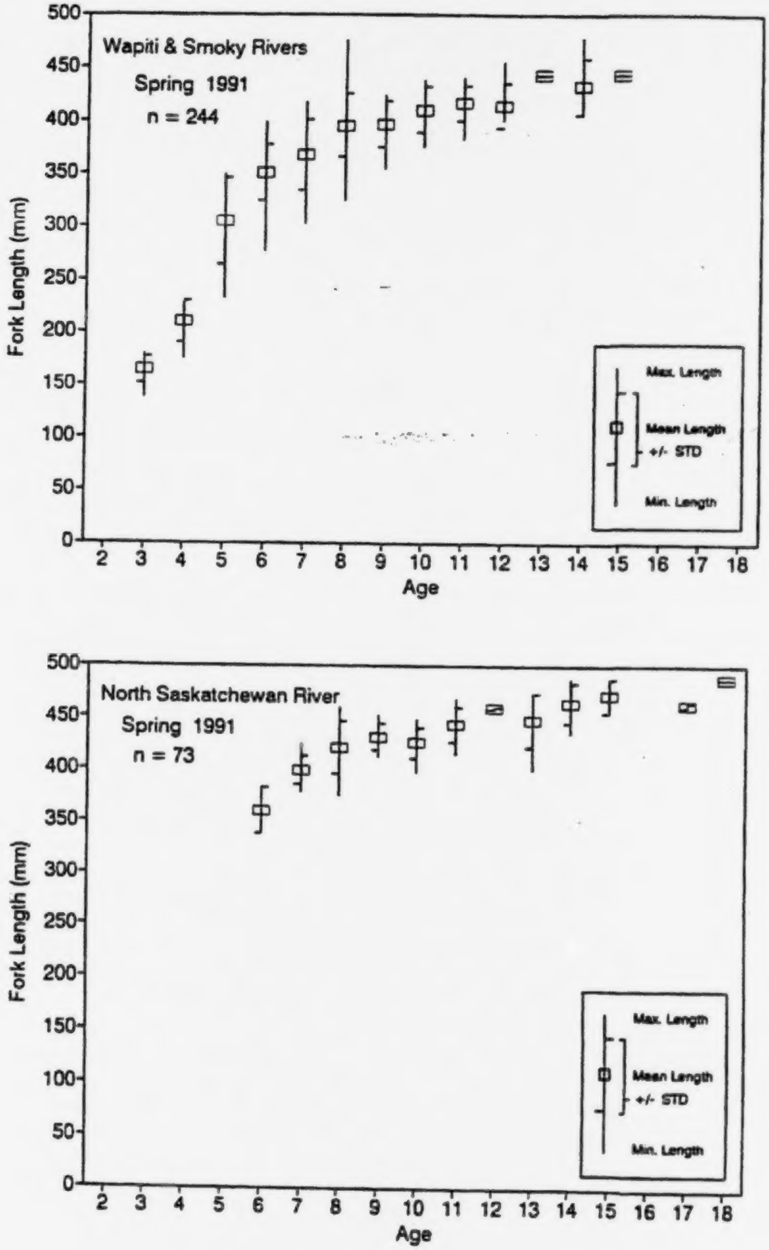


Figure 3. Growth Curves for Rocky Mountain Whitefish from the Wapiti/Smoky and North Saskatchewan River Systems - Spring, 1991.

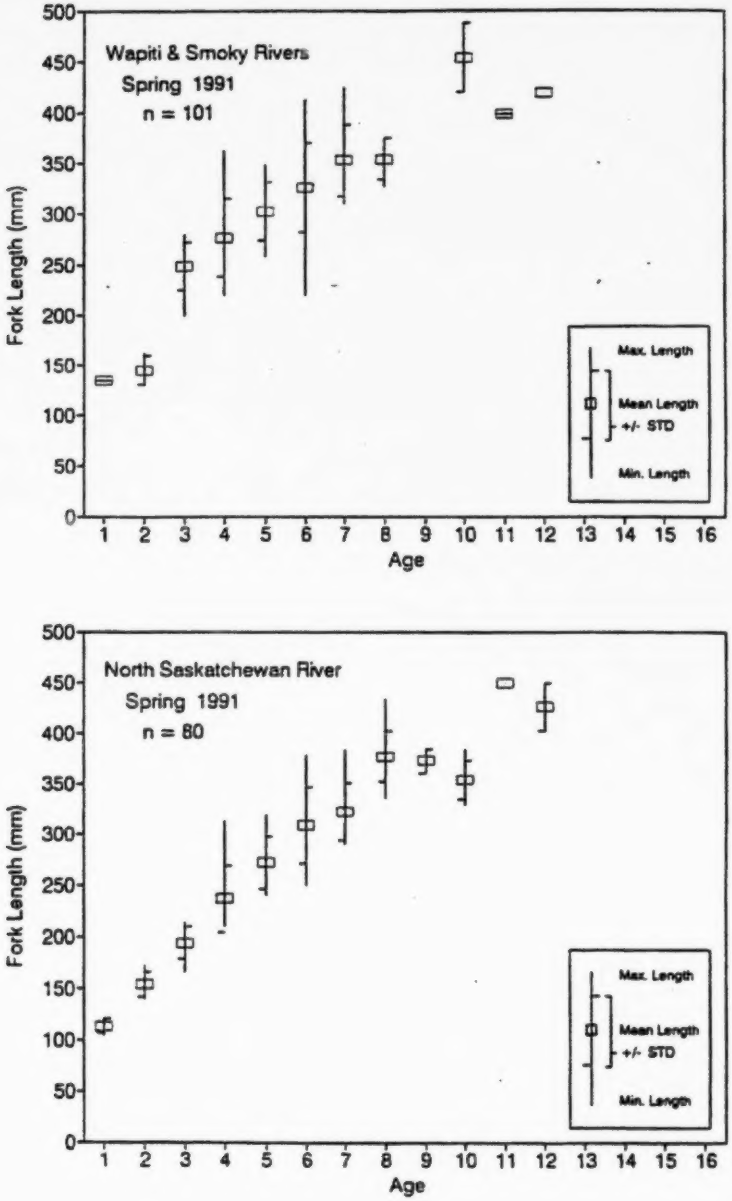
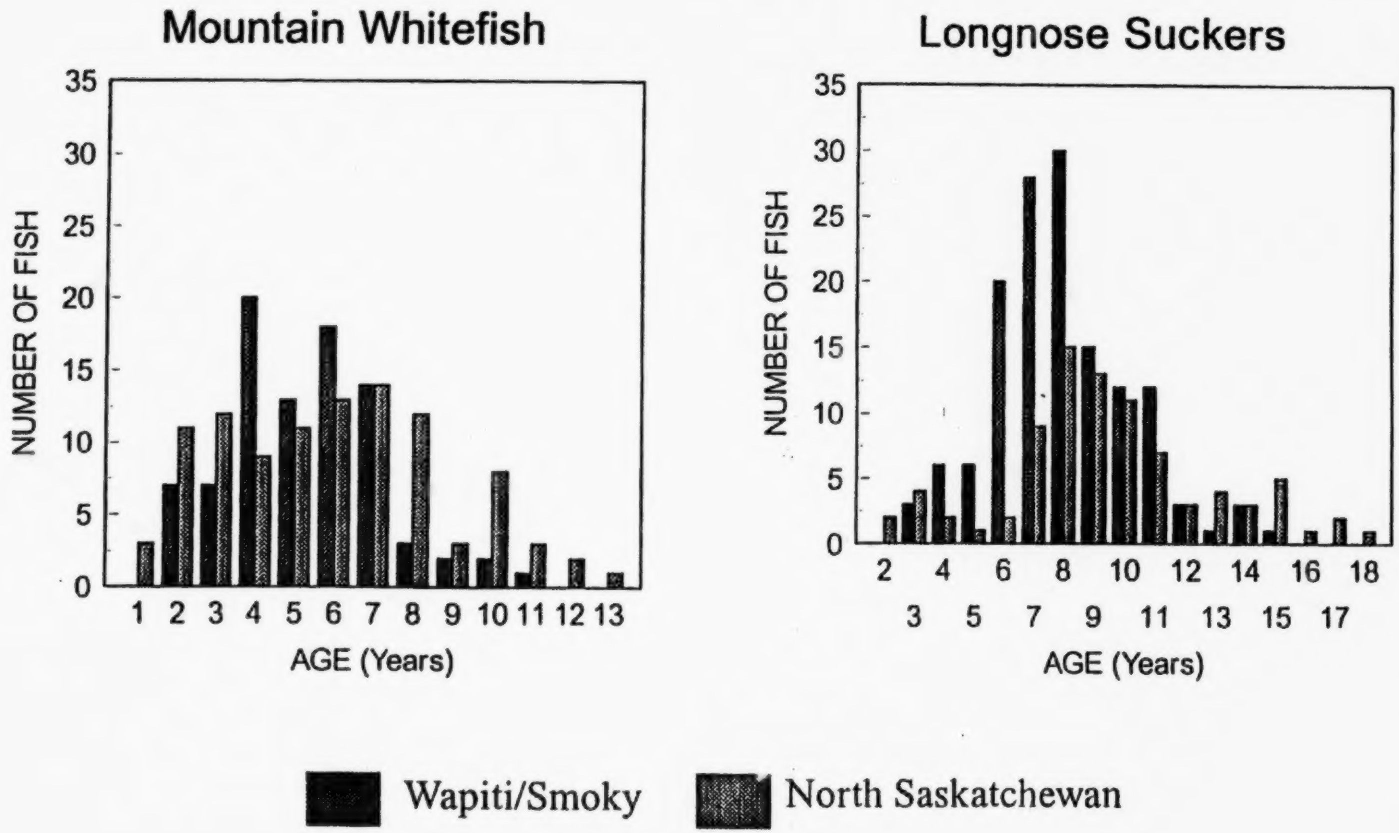


Figure 4. Age Frequency Distributions, 1990-1991.



GENERAL DISCUSSION AND CONCLUSIONS

The fish population parameters examined indicate that there have been no definite exposure-related population impacts in the Wapiti/Smoky river system. Length-weight, growth, age-to-maturity, fecundity, secondary sex characteristics, and gonad size in Wapiti/Smoky fish were not significantly different from fish in the reference river.

Longnose suckers had higher condition factors in the Wapiti/Smoky, probably because of nutrient enrichment. Wapiti/Smoky fish populations had fewer old fish, possibly due to loss of a year-class during a 1:100 year flood in 1972. Recruitment was also severely affected in 1990 during another 1:100 year flood, but had recovered by 1992. Longnose suckers and walleye from 1990/91 did not have significantly different length-weight relationships from fish in 1970/71.

The findings of this study are consistent with previous reviews of BKME at high dilutions (ie. 2% effluent or less) and where significant habitat degradation has not occurred (McLeay 1987, Owens 1991). Significantly, Wapiti/Smoky populations had been exposed to molecular chlorine effluents for 16 years, a minimum of two generations, before chlorine dioxide substitution was begun. Therefore, chlorinated organics at these doses are unlikely to cause environmental impacts at the population level. In fact, the data suggest that natural phenomena are the main source of variation in fish population parameters in both the Wapiti/Smoky and the upper North Saskatchewan.

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EFFLUENT TOXICITY IDENTIFICATION AND REDUCTION.

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ABSTRACT

Toxicity reduction in industrial and municipal effluents can be accomplished following USA Environmental Protection Agency (EPA) guidelines, called "Toxicity Reduction Evaluation" (TRE). The investigation identifies the cause(s) of effluent toxicity and the corrective actions necessary to reduce or eliminate toxicity. Consequently, TREs can be used to meet the needs of dischargers required to eliminate effluent lethality in response to provincial and federal effluent toxicity regulations.

A TRE is designed on a site-specific basis and is conducted in a step-wise fashion to narrow the search for effective effluent toxicity control measures. A major component of the TRE is the "Toxicity Identification Evaluation" (TIE) in which toxicity tests are combined with chemical analyses to identify and confirm causative toxicants.

Properly conducted, the TIE is an extremely effective approach to identifying the cause of effluent toxicity. However, factors such as exposure solution pH drift, the presence of multiple toxicants, and matrix effects can confound data interpretation. Best results are achieved through the joint participation of experienced toxicologists and analytical chemists.

Based on TIE results, investigation of treatability options or process modifications can then focus on reducing or eliminating the specific contaminants of concern.

INTRODUCTION

Effluent regulations for many industrial sectors are currently undergoing revisions at both federal and provincial levels. For example, new regulations have recently been published for the pulp and paper sector under the Fisheries Act and the Ontario Ministry of Environment is developing sector-specific regulations as part of the MISA program. It is generally anticipated that dischargers will be required to eliminate acute lethality to fish and invertebrates. This means that mortality in undiluted (100%) effluent must not exceed 50% in laboratory tests. Facilities discharging effluent that is acutely lethal will be required to identify and implement a means by which they can reduce or eliminate the substances causing the toxicity in their effluent.

The conventional approach to identification of specific effluent toxicants has typically involved comparison of effluent chemical concentrations to the toxic concentrations of each chemical documented in the literature. However, the success of that approach can be limited by a number of factors:

- toxicity data is lacking for many effluent chemicals;
- a correlation between the amount of toxicity and the concentrations of a given chemical does not prove that substance caused toxicity, because other effluent chemicals may co-vary in concentration with the toxicant and may, therefore, be mistaken as the causative agent;

- toxicity data presented in the literature are usually based on tests conducted by exposing organisms to a single toxicant in clean laboratory dilution water. However, in a complex effluent matrix, the influence of other chemicals on the toxicant may increase (e.g., additive or synergistic interactions) or decrease (e.g., antagonistic interactions) toxicity relative to that observed when the toxicant is tested alone; and

- the toxicity of many chemicals is characterized in terms of modifying factors such as pH and hardness, but the maximum range of tested conditions usually reflects those found in receiving waters, whereas the range of pH and hardness found in effluent is much greater.

Consequently, the USA EPA developed an alternative approach to toxicity reduction and five guidance documents were published:

- Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (Fava *et al.*, 1989);
- Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants (Botts *et al.*, 1989);
- Methods for Aquatic Toxicity Identification Evaluations:
  - Phase I Toxicity Identification Procedures (Norberg-King *et al.*, 1991b);
  - Phase II Toxicity Identification Procedures (Mount and Anderson-Carnahan, 1989);
  - Phase III Toxicity Identification Procedures (Mount, 1989).

The TIE guidance manuals listed above were developed to identify toxicants causing acute lethal toxicity of effluents. In light of regulatory initiatives in the USA to also control sublethal toxicity, another manual was recently published giving guidance on conducting sublethal TIEs (Norberg-King *et al.*, 1991a). In addition, guidelines are under development for identification of toxicants in sediments (Ankley *et al.*, 1991).

OVERVIEW OF A TRE

A Toxicity Reduction Evaluation (TRE) is designed on a site-specific basis and is conducted in a step-wise fashion to narrow the search for effective effluent toxicity control measures (Figure 1). This is accomplished by identifying the cause(s) of effluent toxicity and the corrective actions necessary to reduce or eliminate toxicity.

The specific activities involved in conducting a TRE are briefly outlined below.

Acquisition of Relevant Background Data

The TRE should begin by acquiring any information relevant to effluent toxicity. This may include plant process information, influent and effluent physical and chemical monitoring data, effluent toxicity data, and the use of chemicals/materials in processing or treatment. For a sewage treatment plant this may also include data related to sewer use. These data may be used to supplement data generated in the later steps of the TRE and may be useful at that stage to point to potential sources or treatment options.

Evaluation of Facility Operations and Maintenance

This part of the evaluation is performed in order to ascertain whether the facility is consistently well operated and whether effluent toxicity is the result of periodic treatment plant upsets, short-circuiting, or some other operational deficiency that may be causing or contributing to the effluent toxicity. The results of this stage may lead to preliminary strategies for source reduction, improvements in material handling and disposal practises, or substitution or re-cycling of a compound known to be highly toxic.

Toxicity Identification Evaluation

If, after housekeeping and maintenance operations have been optimized, effluent toxicity continues to occur, a Toxicity Identification Evaluation (TIE) is initiated. A TIE is performed in three phases:

- Phase I Characterization;
- Phase II Identification;
- Phase III Confirmation.

Phase I: Characterization

Phase I Characterization is accomplished by conducting a battery of toxicity tests on aliquots of a toxic effluent sample that have each been physically or chemically treated to change specific chemical properties of the effluent. Those treatments that reduce effluent toxicity assist in identifying the physical/chemical characteristics of the toxicant(s) (Table 1). Phase I techniques are generally applied to several (or many) effluent samples to identify whether the toxicant(s) change over time. Phase I information can then be used to decide which chemical analytical methods to use in Phase II.

The number of tests and the nature of several physical-chemical treatments involved in a typical Phase I TIE make it difficult or impossible to use test organisms such as rainbow trout, which require relatively large exposure volumes (e.g., 10 to 20 L). Small organisms such as *Daphnia magna*, *Ceriodaphnia dubia*, or fathead minnow larvae are preferred because exposure volumes of as little as 10 to 50 mL are required. Where effluent toxicity to rainbow trout is a concern, fathead minnow larvae may be used as a surrogate species in early investigative stages of the TIE, but tests must subsequently be performed to confirm that the chemical(s) causing fathead minnow toxicity is/are indeed the same as that/those responsible for rainbow trout toxicity.

Figure 1. Steps in a Toxicity Reduction Evaluation (TRE).

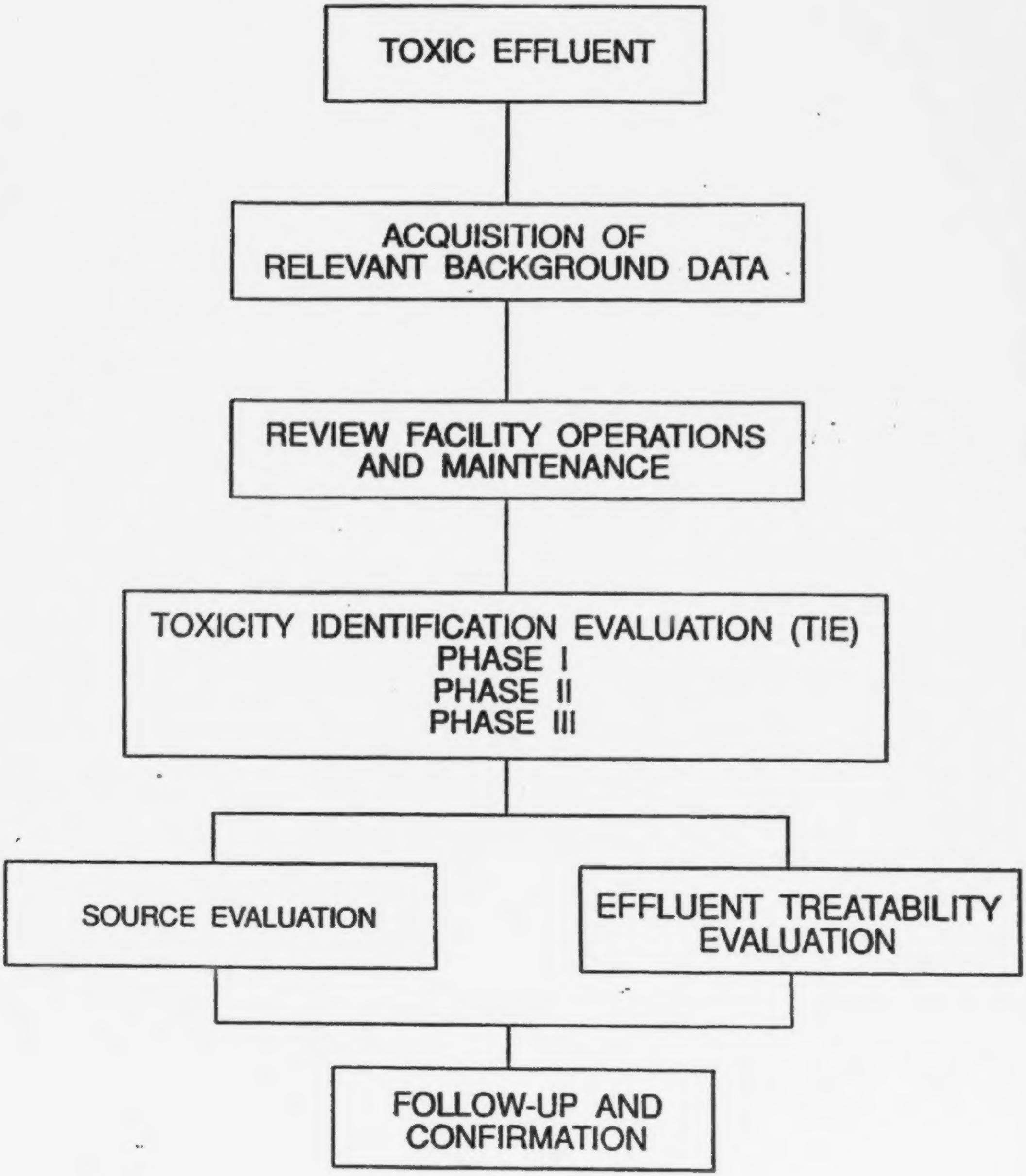

TOXIC EFFLUENT
ACQUISITION OF RELEVANT BACKGROUND DATA
REVIEW FACILITY OPERATIONS AND MAINTENANCE
TOXICITY IDENTIFICATION EVALUATION (TIE)
PHASE I
PHASE II
PHASE III
SOURCE EVALUATION
EFFLUENT TREATABILITY EVALUATION
FOLLOW-UP AND CONFIRMATION

Table 1. Sample Treatments Performed in Phase 1 TIE.

TREATMENT:	AFFECTS TOXICANTS THAT ARE:
Conventional Techniques	
Graduated pH	Ionizable over a pH range of 6 to 9
Aeration	Volatile
SPE (C18 column)	Non-polar organics or metal chelates
EDTA	Cationic metals (e.g., Cd, Cu, Hg, Mn, Ni, Pb, Zn)
Oxidant Reduction	Reduced or chelated by thiosulphate (e.g., chlorine, ozone, Ag, Cd, Cu, Hg, Se)
Filtration	Associated with filterable material
pH Adjustment to 3 or 10[a]	Susceptible to irreversible reaction (e.g., decomposition or precipitation) at extreme pH
Other Techniques	
Activated Carbon	(Non-selective sorption technique that is useful for toxicants that are not effectively altered by other treatments)
Anion Exchange	Anions
Cation Exchange	Cations

[a] Re-adjusted back to circum-neutral pH prior to testing.

Phase II: Identification

Phase II usually involves chemical analyses to identify the specific chemicals suspected to be involved in effluent toxicity. Since some information on toxicant characteristics is available from Phase I data, analytical chemistry is focused on specific chemicals or groups of chemicals of concern, rather than using the "shot gun" approach to analysis that has traditionally been followed.

When Phase I results indicate effluent toxicity is caused by one or more organic chemicals and the effluent contains numerous organic constituents, Phase II may include additional steps to separate effluent constituents from each other before chemical analyses are initiated. For example, a non-polar organic toxicant will be retained when the effluent is passed through a C18 column and solvent extraction will recover the toxicant from the column. However, proceeding immediately to chemical analysis of the extract will usually reveal that numerous other, non-toxic effluent constituents have also been captured in the extract. To further separate the constituents from each other, the extract would then be injected into a high performance liquid chromatograph (HPLC) and aliquots of the extract collected as the sample leaves the instrument. Each aliquot would be tested for toxicity and since each will contain only a fraction of the components present in the original extract, chemical analysis of the toxic fraction will be more likely to identify the specific toxic agent.

Phase III: Confirmation

In Phase III, the identified toxicants are confirmed using a number of procedures, including correlation of toxicity with chemical concentrations, spiking of the toxicant into effluent or dilution water to confirm the toxic level and behaviour of the toxicant in Phase I treatments, toxicity mass balances to ensure all toxicity is accounted for, and comparative testing of additional species.

There is a tendency to shorten or eliminate this final stage since, by this time, an enormous amount of time and money may have been expended and the investigators may be quite convinced of the cause of toxicity (Mount, 1989). However, it is reasonable to expect that physical-chemical manipulations of a complex mixture will, occasionally, introduce artifacts or lead to false conclusions about the toxicants. The specific level of effort that should be expended at this stage should be based on the consequences of arriving at an incorrect toxicant identification. For example, if it is subsequently concluded that toxicity can be prevented by an inexpensive substitution of a less toxic product, there would be low financial risk associated with proceeding without definitive confirmation. On the other hand, the cost associated with a thorough Phase III evaluation, indeed even a full TIE, may be several orders of magnitude less than that required for installation and operation of major capital equipment and considerable savings can be realized by using the TIE to ensure an effective system is selected and implemented.

Based on the results of the TIE, a decision is made on whether to conduct a treatability study on the final effluent and/or conduct a source investigation.

Source Investigation

If the specific causative toxic agents are identified in the TIE, it may be possible to track the toxicant to its source within the plant or municipal sewer grid. At the source, toxicant concentrations may be controlled by such methods as chemical substitution, process modification or elimination, or pretreatment of process streams.

Treatability Evaluation

Toxicity treatability evaluations are conducted to identify possible treatment options that can effectively reduce effluent toxicity and may involve modifications or additions to an existing system. Treatability studies are typically conducted on a bench scale and then on a pilot scale prior to construction of a new treatment system or substantial modification of an existing operation. These studies typically involve treatment techniques similar to those described for the Phase I TIE.

Follow-up and Confirmation

Follow-up monitoring should be conducted to ensure that the toxicity control method selected and implemented was effective in reducing effluent toxicity.

APPLICATION OF TIEs IN NORTH AMERICA

It is estimated that hundreds of TIEs have been conducted in the USA during the past five years that incorporated many elements of the USA EPA approach. A number of state environmental agencies have written TIE requirements into discharge permits. While the majority of TIEs performed in the USA to date have addressed acute lethality, a number of American dischargers that have resolved the lethal toxicity of their effluents are now applying a similar TIE approach to identify the constituents causing sublethal effluent toxicity. To date, Canadian industries have initiated relatively few TIEs, but presumably, as new effluent toxicity regulations come into effect, there will be a greater need for such studies.

The applicability of the TIE guidelines need not be restricted to the end-points described by the EPA manuals. A particular strength of the guidelines lies in the philosophical approach, since the same approach can be applied to solving the chemicals causing responses other than lethal and sublethal toxicity. BEAK is currently adapting the TIE approach to identify tainting compounds in fish by conducting taste tests on various extracts of tainted fish samples. Similarly, the Ames test or SOS-Chromotest could be substituted for aquatic toxicity tests to identify mutagenic substances in effluents.

SPECIAL CONSIDERATIONS

Although the EPA guidelines have been enormously helpful in assisting dischargers identify the cause of effluent toxicity, they are not, nor can they ever be, rigid protocol documents. While the methods for conducting Phase I characterization are relatively well developed, only limited interpretive guidance for that and subsequent phases has been provided, since the combinations of potential results are as numerous as there are dischargers. Knowledge as to how specific chemicals can be expected to behave throughout the specified battery of Phase I tests is being acquired for common effluent toxicants (e.g., ammonia, copper), but even these chemicals may behave differently in the unique matrix of a given effluent.

Important considerations to bear in mind when conducting TIEs are too numerous to include in this discussion, and are well-described in TIE training courses provided in the USA from time to time. However, a few experiences from TIEs performed by BEAK can be used to illustrate some of the challenges and complexities that can be involved.

Marginally Lethal Effluents

Phase I TIE characterization is based on identifying one or more treatments that reduce toxicity relative to the toxicity observed in an untreated sample. When the LC50 of an untreated sample is close to 100%, reduction in toxicity may be interpreted as a successful treatment or it may simply be within the range of response variability that should be expected in replicate tests of that sample. This can necessitate characterization of a greater number of effluent samples over time than would otherwise be required to verify that an observed response is truly an effect of the treatment and not random variability.

Infrequently Lethal Effluents

When effluents are only occasionally lethal, it may take years to capture a sufficient number of lethal episodes to characterize toxicity. This necessitates considerable patience, not only on the part of the discharger paying for the work and the consultant conducting the work, but also from the regulators anxious to enforce consistent compliance with toxicity limits. If toxicity is very infrequent (e.g., less than 20% of samples are lethal) or is the result of a single, short-term episodic event (e.g., a spill or plant upset) a TRE is probably not appropriate (USA EPA, 1991).

Achieving Compliance with More than One Species

It appears that some, if not all, effluent dischargers in Canada will ultimately be required to discharge an effluent that does not cause acute lethality to a selected fish and an invertebrate test species. In Ontario, for example, it is likely that rainbow trout and *Daphnia magna* will be the two species used in compliance monitoring.

When an effluent consistently causes toxicity to both rainbow trout and *Daphnia magna*, it is tempting to assume that whatever is causing toxicity to one species is probably also the cause of toxicity to the other species. The likelihood that this is the case can quickly be determined by reviewing LC50 data for all samples where both species were tested. If the ratio of trout to *Daphnia* LC50 is relatively constant among samples, the assumption that the same chemical causes toxicity of both species may be true; otherwise, the chemical or chemicals causing toxicity to each species is quite likely different and a separate TIEs may be required for each species.

Exposure pH Drift

The pH of exposure solutions tends to drift during toxicity tests usually as a result of CO_2 loss causing a shift in the carbonate buffer system (Mount and Mount, 1992). If toxicity is caused by a toxicant that is pH-sensitive (e.g., ionizable chemicals, as well as some metals), differences in pH among tests can greatly confound interpretation. The data presented in Table 2, would, at first, lead one to suspect that sample purging resulted in toxicity reduction. However, exposure pH in the nitrogen purged aliquots showed less tendency to drift upwards than the non-purged aliquots of sample. When organisms were subsequently exposed to the same purged and non-purged samples adjusted to different exposure pHs, both showed the same toxicity response pattern (Table 3), indicating that it was the difference in exposure pH in the original purging test (Table 2), not the purging itself that resulted in mortality of fish.

Table 2. Effect of Sample Purging Versus Exposure pH on Trout Toxicity

Treatment	Nominal Exposure pH	Percent Mortality	Measured pH
None	6	0	5.9 - 6.8
	8	100	7.9 - 8.7
Nitrogen Purge	6	0	5.9 - 6.5
	8	0	7.9 - 8.1

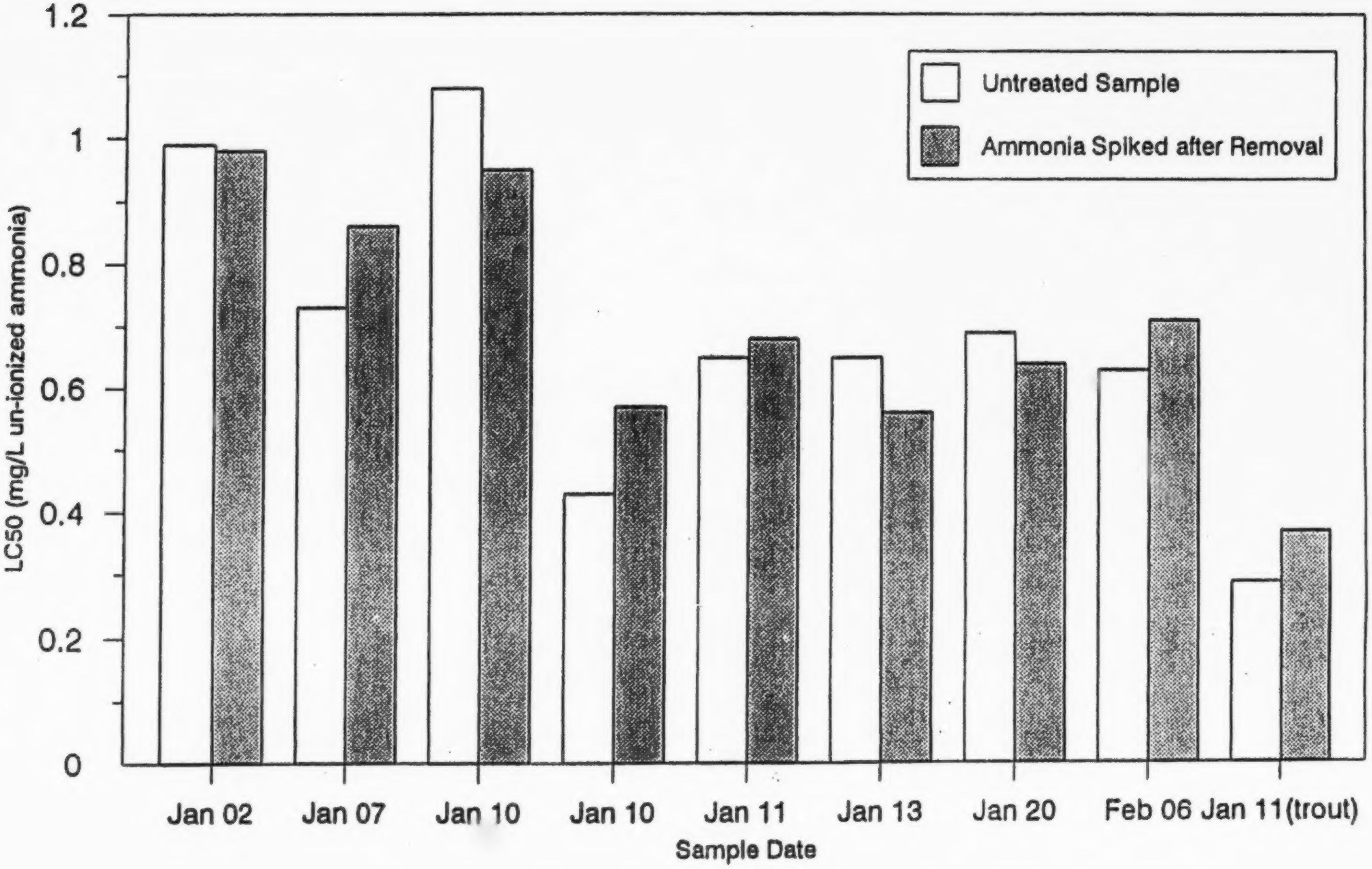
Table 3. Graduated pH Test

	Percent Mortality	
Exposure pH	**No Treatment**	**Nitrogen Purged**
6	0	0
7	0	0
8	0	0
9	100	100

Interaction of Toxicants with Other Effluent Constituents

In effluents where the toxicants interact with other effluent constituents, data interpretation throughout the TIE can be difficult. In the case of additive or synergistic toxic

Figure 3. **Fathead Minnow LC50 of Effluent Samples Expressed as Un-ionized Ammonia Concentration.**



required to reduce contaminant concentrations, the cost of arriving at an incorrect conclusion may be considerable. However, in a TIE, possible causes or sources of toxicity are eliminated, step by step, until an appropriate solution or control method is identified. The cost of even a complex and intensive TIE can greatly outweigh that of installing inappropriate remedial process equipment or treatment systems. EPA experience has shown that unnecessary delays and expenditures in achieving reduced effluent toxicity are avoided by using the TRE approach to build a sound scientific and engineering basis for selection of a control method (USA EPA, 1991). The key to a successful TIE lies in the joint participation of experienced toxicologists and chemists.

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ISOLATION AND IDENTIFICATION OF TOXIC SUBSTANCES PRESENT IN AN INDUSTRIAL EFFLUENT SAMPLE.

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In the fall of 1991, the effluent discharge to the Red Deer River from the Novacor Industrial facility at Red Deer degraded in toxicity, such that the LC_{50} for the 96 h rainbow trout acute bioassay was less than 100%. Preliminary investigations conducted by HydroQual Laboratories revealed that the effluent was comprised of three major waste streams and that the waste stream from cooling tower Ell was the most toxic. HydroQual Laboratories Ltd., in association with Enviro-Test Laboratories, was directed by Novacor to conduct a formal Toxicity Identification Evaluation (TIE) in order to identify the causative agents. Procedures used were modifications of those developed by the United States Environmental Protection Agency. Toxicity was evaluated using three procedures, namely, Microtox, Daphnia, and Fathead minnow larvae mortality. Toxicants were effectively concentrated from effluent and cooling tower waste streams using solid phase extraction procedures and were isolated by stripping the solid phase adsorbent with 75% methanol: 25% water. Chemical derivatization of this fraction followed by analysis using gas chromatography/mass spectrometry, revealed the presence of mono-and dibrominated indolones in effluent and the Ell cooling tower waste stream. Confirmation of these toxicants was performed by initiating process changes and performing TIE evaluations of the resulting effluent from the cooling towers.

BEYOND THE TIE: A CASE STUDY OF HIGHLY-POLAR ORGANIC CONTAMINANTS IN GROUNDWATER.

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Groundwater flowing into the North Saskatchewan River at Edmonton, AB, was contaminated by highly toxic organic wastes from an industrial site. The contaminant mixture was complex and extremely water soluble, making extraction and identification of the most toxic fraction very difficult. A combination of spinning band distillation, high pressure liquid chromatography, chemical ionization gas chromatography/mass spectrometry (GC/MS) and electron impact GC/MS were successfully used to identify 28 compounds in the most toxic fraction. This list was shortened by evaluating the toxicity of the compounds. Toxicological information including information on the effect on target organs, pathological examinations and behaviourial studies were used to identify allyl alcohol as the primary cause of the toxicity to rainbow trout. Verification included acute bioassays and pathological examination. Allyl alcohol was not the cause of the Microtox toxicity which was due primarily to formaldehyde and formic acid. The results of this study emphasize the necessity of using multiple-species testing.

CASE HISTORY - TOXICITY ALARM. D. Hogan and J. Retallack, Novacor Chemicals Ltd., P.O. Box 5006, Red Deer, AB, T4N 6A1, (403) 342-8611.

ABSTRACT

In the late fall of 1991, Novacor's world scale petrochemical complex experienced a toxicity alarm, the first of its kind since start-up, approximately 14 years ago. Fish exposed to a normal toxicity test were stressed within 2 hours and showed 100% mortality after 24 hours. This paper is a history of events leading up to, during and after the toxicity alarm.

The major effluent sources were three Cooling water systems. Although these sources are well characterized, the event causes were not immediately clear. Initial toxic screening indicated that one system was very toxic, another was only moderately toxic and the third was not toxic at all. All three systems utilized the same chemical treatment program - stabilized phosphates with minor variations. The cooling system that was most toxic operated at 10-12 cycles, had three chemicals for biocide control and had three makeup streams. The system classed as moderately toxic operated at 6-8 cycles with two chemicals for biocide control and had two makeup streams. The third system, initially screened as nontoxic, operated at 10-12 cycles with two biocide chemicals and had only one makeup stream.

Toxic and nontoxic system characteristics were compared. An in-depth modified Toxicity Identification and Evaluation (TIE) program was then performed to identify and evaluate the cause of the toxicity alarm for future prevention. The most probable causes of toxicity were identified by elimination. Work now proceeds to monitor and improve our ability to control the identified causes of toxicity.

INTRODUCTION

For the petrochemical industry to remain as a dynamic publicly supported enterprise, it must maintain a responsible care attitude beyond suspicion or reproach. Industry is accountable for any and all environmental impacts resulting from its activities. The challenge for the nineties is to demonstrate this responsible care attitude by being proactive in virtual elimination based on valid risk assessment. It may be impossible or unnecessary to achieve an ideal zero discharge state.

However, an achievable level of discharge whereby the quantity and quality of the effluent does not have an adverse environmental impact is possible. Zero discharge of all effluent may be impossible, but the task of approaching zero discharge and ensuring a zero adverse environmental impact is never ending. Virtually all of the time, our proactive approach results in positive gains. This paper is a case history of a situation where best intentions to improve a system failed due to unforeseen circumstances. Efforts to improve environmental impact resulted in a more severe temporary situation.

SYSTEM DESCRIPTION

Joffre Site

Novacor Chemicals Ltd. located near Joffre, Alberta produces ethylene and polyethylene. Ethylene is the primary building block for the plastics and petrochemical industry. To make ethylene, ethane is extracted from natural gas and imported onto the Joffre site for conversion to ethylene at our two ethylene plants. The ethylene leaves the site via pipeline. Much of the ethylene is shipped to customers in the Edmonton area. Ethylene is also "exported" to the Novacor polyethylene complex located within the petrochemical site.

Ethylene I, commissioned in 1979, produces about 0.6 billion kilograms per year. Ethylene II, commissioned in 1984, produces about 0.8 billion kilograms of ethylene per year, making it the largest ethylene plant in Canada. The ethylene process involves vaporizing and pyrolysing the ethane feedstock at very high temperatures followed by compressing/refrigerating/distilling toward the final ethylene product.

The polyethylene plant, commissioned in 1984, has an annual production of about 0.5 billion kilograms of polyethylene. The plant obtains the primary feedstock ethylene from the Ethylene facility. The ethylene is reacted with comonomers, butene or hexene to produce the polyethylene product. This product, in pellet and granular form, is shipped via rail and sold domestically and internationally.

Water Withdrawal System

Water for the Novacor site is extracted from the Red Deer River. The river water pumphouse is equipped with two large mechanical screens and pumps. The river water pumps, which are capable of delivering 684 m3/h each, pump to a desilting pond. The desilting pond has a holding capacity of 20,900 m3 of water. The desilting pond is connected to a high lift pond which has a capacity of 413,000 m3 of water. A high lift pond pumphouse is equipped with three pumps each capable of delivering 900 m3/h. The high lift pumps supply all the site water needs for potable, demineralization, cooling, utility and firewater protection systems.

Demineralization System

The demineralization system provides all the boiler feed water for the site. The primary components are three 50% strong acid cation exchangers, a decarbonator and three 50% strong base anion exchangers. All exchangers utilize water block counter-current regeneration. The average service rate is 100 m3/h. The units are regenerated on a cation/anion train basis to provide self neutralization. This neutralized regenerant waste averages 10 m3/h and this is approximately 9% of the total liquid effluent waste generated by the site.

Cooling Tower Systems

The Joffre site processes results in excess heat. Although some heat energy is utilized at the Novacor greenhouse operation at Joffre, the bulk of this excess heat load must be removed via large evaporative cooling systems. Refer to Table 1. Each of the three plants - Ethylene I, Ethylene II and Polyethylene - utilize large crossflow cooling water towers. The system water volumes for Ethylene I and Ethylene II are 7500 m3 each. Polyethylene has a system water volume of 1000 m3. The circulation rate for Ethylene I and Ethylene II are 26,000 m3/h and 22,000 m3/h while Polyethylene is 5,400 m3/h. The average temperature drop across all three towers is 12°C. The combined blowdowns from these towers constitute over 91% of the site effluent.

Table 1. Cooling Systems Design Basis

Cooling System	Ethylene I	Ethylene II	Polyethylene
Manufacture	Ecodyne	Marley	Marley
No. of Cells	12	10	4
Recirculation Rate (m3/h)	26,000	22,000	5,400
Avg. Temperature Drop	12°C	12°C	12°C
Evaporation Rate (m3/h)	393	333	74.8
Cycles of Concentration	10	10	10
Blowdown Rate (m3/h)	43.7	37	8.3
Makeup Rate (m3/h)	437	370	83
System Volume (m3)	7500	7500	1000
Side Stream Filtration	Yes	Yes	Yes

Waste Effluent System

Facilities are provided to collect and impound all plant waste waters in two effluent ponds. Each pond is designed for a holding capacity of 22,074 m3 or 5 days effluent accumulation resulting from the cooling tower blowdowns and demin plant regenerations.

A special recycle distributor system for each pond is provided to ensure adequate mixing and homogeneity of the pond contents. Three pumps are provided in the effluent pumphouse. One large pump is used to recycle or mix the contents of the ponds. Two smaller pumps are used to pump the effluent to the Red Deer River. After the effluent pond contents are analyzed to ensure licence compliance, two smaller pumps are used to pump the effluent to the Red Deer river. The rate is dictated by the waste discharge licence parameters and volume bottleneck requirements.

The m3/h flow values in Figure I were typical flows measurements at the time of the toxicity incident.

TOXIC CONDITIONS

On November 25 1992, Novacor chemicals was notified by a contract lab that an effluent water sample collected on November 19 had failed a rainbow trout acute bioassay test. This was the first happening of its kind for the history of the site. In accordance with the licence to operate, pursuant to the clean water act, a second sample sent on the 25th, failed the same test. The lab technician who conducted the bioassays observed that within the first two hours the test fish turned dark and started swimming slightly on their side. This stressed condition became noticeably worse as time went on resulting in 100% mortality after 24 hours.

Formation of Task Force

Confirmation of the toxicity condition led to the formation of a task force headed by Utilities and Olefins Division (U&OD). In addition to personnel from U&OD, the ethylene units and environmental services, the task force included outside consultants with relevant expertise.

U&OD is the operating unit responsible for running all water related functions such as extraction, pretreatment and distribution. This support service, to the ethylene and polyethylene operating units, also includes collection and treatment of all aqueous effluent streams for recycle and discharge to the Red Deer River.

Task Force Mandate

The task force focused their activities to solve the following:

- What can be done immediately to mitigate the problem?
- Where or what is the source of the toxicity?
- This is the first occurrence ever. What is different?
- Discover and stop the activity that's causing the toxicity.
- Set up measures to prevent future re-occurrence.

Short Term Solution

By the time the toxic condition was confirmed all available effluent storage had been used up. Approximately 20,000 m3 of effluent waste water was pumped to a large retention pond. Through this activity, the discharge of potentially toxic effluent was avoided until quality confirmation. This retention pond is normally used as a reservoir for site storm water drainage and process area washings. The contents of this retention pond is recycled as a makeup stream to Ethylene I cooling tower.

This routing of effluent to the retention pond however, was only a short term solution for the following reasons. Ethylene I cooling tower is designed to operate at 10 to 12 cycles of concentration. The evaporative effect of the towers will cause all non-volatile organic and inorganic compounds to cycle up in concentrations. For example, if the makeup to a cooling tower contains 40 ppm of any non-volatile compound then after evaporation, at 10 cycles of concentration, the tower bulk water will contain 400 ppm of that non-volatile compound. All non-volatile constituents in the makeup water will concentrate by a factor of 10. The effluent pond is typically extremely high in dissolved solids, especially inorganic hardness salts. Because of the high salt content, it cannot be used as CT makeup. Transferring the effluent to the retention pond, which is used as makeup to Ethylene I cooling tower, causes an increase in Ethylene I cooling tower blowdown to the effluent system.

Finding Source of Toxicity

Four subsystems contribute to the aqueous effluent system in Figure 1. The combined blowdowns from the cooling towers account for over 90% of the aqueous effluent. Ethylene I CT was the largest contributor at 51 m3/h. Ethylene II CT was next at 39 m3/h while the blowdown from Polyethylene CT was 8 m3/h. The regenerant effluent from the demin plant accounted for 10 m3/h.

The initial toxicity investigation, utilizing test fish and Microtox, indicated that the source of the toxicity was in the combined effluent of the cooling water systems from Ethylene I, Ethylene II and Polyethylene. The pH neutralized effluent from the demineralization system did not appear to be the toxicity source. Further cooling water characterization revealed that the cooling water blowdown from Ethylene II was the most toxic. The cooling water blowdown from Ethylene I was less toxic than Ethylene II, while that from Polyethylene was not toxic at all.

The makeup water for the cooling systems is lime softened river water, storm drainage, and process quench water. All of the makeup to Polyethylene CT (83 m3/h) is lime softened river water. The makeup for Ethylene I is 437 m3/h, 50% of which is lime softened river water, 30% is recycled storm drainage and 20% is process quench water. Ethylene II requires 370 m3/h. About 80% or 296 m3/h is lime softened river water. The remaining 20% or 74 m3/h is plant process effluent water (quench).

"Stabilized Phosphate" is the chemical treatment program employed by all three cooling tower systems. This program prevents scaling and corrosion damage to heat exchangers and other equipment in contact with cycled up cooling water. Zinc is used in the ethylene cooling systems to "beef up" the phosphate treatment. Zinc is not used in the Poly system. Biological fouling is prevented by the continuous addition of oxidizing biocides such as chlorine or bromine. Occasionally, non-oxidizing biocides are used on a shock dosage basis for biological control. Ethylene I and Polyethylene used chlorine as its oxidizing biocide. Ethylene II used a combination of chlorine and bromine as its oxidizing biocide.

Table 2. Variations in Cooling Systems

	Polyethylene	Ethylene I	Ethylene II
Toxicity	Not toxic	Moderate	Very
Makeup			
River water	100%	50%	80%
Storm drainage		30%	
Process quench		20%	20%
Treatment			
Phosphate	yes	yes	yes
Chlorine	yes	yes	yes
Zinc	no	yes	yes
Bromine	no	no	yes

What Was Different?

The task force looked at what was different before and after the toxic condition. They also studied the differences between the systems and the rationale of these variations. Previous to April 1992, the chemical treatment programs for the cooling systems in the ethylene and polyethylene plants were "all organic phosphate" based. From April to the present the programs are "stabilized phosphate" based. The decision to change from "all organic phosphate" to "stabilized phosphate" was one of performance. The "all organic phosphate" programs did not perform satisfactorily at the Joffre site. Switching to the "stabilized phosphate" would also allow for higher concentrations in the cooling towers.

Up to August 1991, the ethylene cooling towers had been operated at 7 to 8 cycles of concentration. From August on, the cycles in Ethylene II had been slowly increased to 12 cycles. The Ethylene I tower had been increased to 9 cycles of concentration. There was no significant change in the Polyethylene tower. It had always operated at approximately 10 cycles of concentration.

Higher cycles of concentrations have several major advantages: - less makeup or river water extraction - less blowdown or less effluent discharge and less treatment chemical usage.

The ethylene plants used process quench water as part at their cooling tower makeup requirements. Process quench water contains trace amounts and varying types of hydrocarbons. The polyethylene tower does not use any hydrocarbon containing makeup water. This has been the design basis for all three plants since start-up. Ethylene I is the only cooling system that includes storm drainage from the retention pond as a part of makeup. Only on rare occasions of abnormally high rain storms, will Ethylene II and Polyethylene makeup with water from the retention pond.

The ethylene plants used zinc to supplement their chemical treatment programs. Zinc provides additional cathodic protection for mild steel components. The process quench water used as makeup in the ethylene systems has historically put additional stress on the chemical treatment programs. Polyethylene does not use process quench water, there was no need for additional protection by using zinc.

Ethylene II was the only cooling system that used a combination of chlorine and bromine as an oxidizing biocide. Ethylene I and Polyethylene used chlorine alone. Prior to bromine addition, Ethylene II was not able to feed enough chlorine to maintain a free residual in the cooling system. Microbiological fouling was a chronic problem. Rather then increase the chlorine feed system, it was decided to supplement with sodium bromide, a safer handling, liquid halogen compound. The byproducts of bromine compounds are reported to have a shorter half-life than those of chlorine.

Preliminary Conclusions

The polyethylene cooling water blowdown did not contain a toxic substance. Therefore, the individual effect or combined effects of the "treated river water makeup", "stabilized phosphate program" and the oxidizing agent "chlorine" could be ruled out as the cause or causes.

Toxicity to rainbow trout was detected in the blowdowns from both Ethylene plants. Therefore the factors that cannot be ruled out as contributors to the toxicity are "storm drainage makeup", quench water makeup", "zinc treatment" and "bromine oxidation".

Increasing the cycles of concentrations increased the concentration of the toxicity causing substance. The most toxic system was Ethylene II which ran at the highest cycles of concentration.

Both ethylene plants exhibited toxic symptoms. Both ethylene plants use process quench water which contains a wide variety of trace hydrocarbons ranging from C4s to C18s.

The presence of hydrocarbon compounds in a chlorine environment will result in the production of halogenated hydrocarbons. The toxicity of halogenated hydrocarbons will increase with increasing cycles of concentration.

Bromamines are more toxic then chloramines. Brominated hydrocarbons are more toxic then chlorinated hydrocarbons.

Toxicity Identification and Evaluation Study

In late December Hydroqual Laboratories Ltd., in association with Enviro-Test Laboratories, was directed by Novacor to conduct a formal Toxicity Identification and Evaluation (TIE) using the United States Environmental Protection Agency (USA EPA) protocol (USA EPA 1991). The TIE protocol is a systematic laboratory procedure designed to identify the toxic constituents within a complex wastewater. The presence and potency of the toxicants in the samples are detected by performing various sample manipulations and by using other aquatic organisms to track the changes in toxicity caused by these manipulations. This allows separation of the toxicants from the other inorganic or organic constituents in the sample. This in turn simplifies the analytical identification of the toxic compound(s).

Hydroqual's conclusions were as follows:

The toxic component of Novacor's wastewater effluent can be isolated in the 50% and 75% methanol/water elutriates from a C18 solid phase extraction. More toxicity is recovered in the 75% compared to the 50% elutriate.

GC/MS analysis of the organic constituents in these methanol elutriates indicate the toxic compounds are aromatic compounds which contain nitrogen and bromine. In particular, they are likely bromine and methyl substituted indolines.

These compounds appear to be formed in the cooling towers following the direct addition of bromine as an anti-fouling agent.

Actions Taken

Three large filter units were charged with Granular Activated Carbon (GAC). All cooling tower blowdown is filtered through this media enroute to the effluent holding ponds. A spare charge of GAC is kept on hand to provide a change out if required.

The practice of using sodium bromide to supplement chlorine use was stopped.

The cycles of concentration in the ethylene cooling towers were immediately lowered to 1990 levels.

Equipment was purchased and an in-house capability for doing Microtox was developed. A new operating procedure calls for Microtox testing on every effluent pond prior to discharge.

Samples are collected and sent to a government approved bioassay lab for testing at least once per month and more frequently as required.

A slipstream of the effluent is passed through an aquarium containing rainbow fry prior to and during discharge. Any abnormalities will cause an immediate effluent system shutdown.

Conclusion

As stated in the introduction, this case history is an excellent example of good intentions producing unacceptable results.

Good Intentions

Increasing the cycles of concentration had the desired effect of reducing waste effluent volume and less chemical treatment material to the Red Deer River. This also resulted in reducing the make up requirement from the river.

Introducing a sodium bromide salt to Ethylene II decreased the quantity of chlorine gas required onsite. The byproducts of bromine have a shorter half-life than those of chlorine.

Reusing hydrocarbon contaminated process quench water as make-up to the cooling system is a resourceful recycling method.

Unacceptable Results

We now know that, when we increased the cycles, we also increased the concentration of hydrocarbons. Given all the relevant facts and the results of TIE, one can easily postulate that the combination of high cycles, hydrocarbon and bromine resulted in the formation of aromatic bromamines. These aromatic bromamines are capable of causing the toxic condition experienced.

APPLICATION OF THE SHORT-TERM CHRONIC TESTS IN TOXICITY IDENTIFICATION EVALUATION PROGRAM.

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A primary use of toxicological data generated during effluent testing is to establish safe limits for the wastewaters entering the aquatic environment. Effluents, whether from municipal or industrial sources, contain thousands of constituents which may potentially cause toxic effects to aquatic organisms. The toxicity of effluents to aquatic organisms is measured by standard methodology. Although long recognized as a rapid technique to measure toxicity of wastewaters, acute and short-term chronic testing provides a starting point for the hazard assessment of discharged wastewaters. To assist in separation and identification of toxicity in wastewaters the toxicity identification evaluation (TIE) program was developed. Identifying the source of toxicity in wastewater is based on the principal of sequential removal of the constituents coupled with a toxicity test on the fractionated effluent. The TIE programs conducted extensively over the past nine years used exclusively the acute toxicity test methods. The acute TIE procedures were not effective with samples exhibiting marginal toxicity, therefore the Environmental Protection Agency (EPA) recently developed a methodology to identify chronically toxic constituents in wastewaters. The overall objective of this study was to identify and characterize the toxic constituents in two process waters which proved chronically toxic to *Ceriodaphnia dubia*. The presentation will also focus on the difficulties encountered during this program which are a combination of inadequate fractionation methodology as well as limited choice of applicable test methods.

RESOLVING COMPLEX MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS IN AMERICAN LOBSTER (*Homarus americanus*) CAPTURED IN SYDNEY HARBOUR.

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A study carried out in 1991 confirmed the persistence of high polycyclic aromatic hydrocarbon (PAH) concentrations in lobster captured in South Arm of Sydney Harbour, Nova Scotia. In addition to routinely monitored PAHs, a multitude of complex PAH components were evident in the 1991 study. PAH, alkylated PAH, and sulfur-, nitrogen-, oxygen-containing heterocyclic aromatic hydrocarbons (PASH, PANH and PAOH respectively) in lobster were resolved using the techniques of gas chromatography-mass spectrometry and retention indices. Difficulty with isolating PANH from lobster using the current methodology and the carcinogenic components identified in the study will be discussed.

TOXICITY OF SCRAP TIRE MATERIALS TO SELECTED AQUATIC ORGANISMS.

J.S. Goudey, HydroQual Laboratories Ltd., Calgary, AB, (403) 253-7121; B.A. Barton, Environmental Management Associates, Calgary, AB.

Scrap automobile tires are widely used as artificial reefs to enhance fish habitat. Tires have been considered safe and biologically non-polluting despite the fact that they contain a number of leachable toxic compounds such as zinc and polynuclear aromatic and petroleum hydrocarbons. In order to assess potential ecological risks, we conducted a battery of tests on scrap tires with a number of different aquatic organisms (trout, *Daphnia*, *Ceriodaphnia*, bacterial bioluminescence, *Selenastrum*). Pieces of 6 tires were incubated in dechlorinated tap water at 15°C (200g of tire per litre of solution). The amounts of material released within a 24h extraction period were acutely toxic to aquatic life. Differences between species sensitivities and the toxicities of individual tires were noted. After 65 successive 24h extractions of tire pieces with 20L of dilution water (200g tires per litre of solution), the tires continued to release material toxic to trout, *Daphnia*, and bacterial bioluminescence.

TOXICITY OF LEACHATE FROM ASPEN WOOD.

J.S. Goudey, HydroQual Laboratories Ltd., Calgary, AB, (403) 253-7121; B.R. Taylor, Environmental Management Associates, Vancouver, BC; B. Carmichael, British Columbia Ministry of Environment, Prince George, BC.

Winter logpiles of aspen (*Populus* spp.) wood sometimes produce a dark watery, toxic leachate, locally known as blackwater, during and after spring melt. Because of the threat to aquatic ecosystems from blackwater produced during aspen harvesting, a laboratory study was undertaken to elucidate the nature and strength of aspen leachate toxicity and the chemical composition of the leachate. Fifty kilograms of aspen wood chips were leached in 200L of dechlorinated tapwater for 30 days. The final leachate was amber in colour, anoxic, and had a high BOD (>2600 mg/L), contents of organic matter (2480 mg/L) and phenols (30 mg/L). The leachate was highly toxic to trout and other organisms (EC50/LC50's <1% sample strength). No change in toxicity was noted during storage at 5 or 20°C. However, aeration turned the sample black and reduced the toxicity. A modified toxicity identification evaluation (TIE, Phase 1) was conducted to characterize the toxic constituents present in the leachate.

TOXICITY OF MUNICIPAL WASTEWATER TO TWO SPECIES OF FISH, THE CLADOCERAN *DAPHNIA MAGNA* AND THE MOLLUSC *ANODONTA GRANDIS*.

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Abstract

The acute and chronic toxicity to rainbow trout (*Oncorhynchus mykiss*) and a cladoceran (*Daphnia magna*) of wastewater from two municipal treatment plants situated in Edmonton, Alberta, were determined. Fathead minnow (*Pimephales promelas*) and a mollusc (*Anodonta grandis*) were also used in acute toxicity tests. The two treatment plants used a mechanical-biological means to process wastewater, and did not chlorinate their effluent. Prior to implementation of toxicological procedures, the wastewater samples were manipulated to reduce ammonia-N concentrations. Influent and effluent from both plants were generally non-acutely toxic (LC_{50} ≥100%) to the four test species, regardless of the time of year when the samples were collected. Histopathological evaluation of fish acutely exposed to influent and effluent revealed minor disorders associated with the skin. Exposure of *Daphnia magna* for 21 d to effluent resulted in a statistically significant increase in the production of neonates, suggesting an antagonistic interaction between enhanced food supply and the presence of potential toxicants. Mortality of *Daphnia magna* during the 21-d exposure was significantly less or similar to controls at all effluent concentrations. Rainbow trout, exposed to effluent for 28/29 d, showed no change in mortality, body weight or fork length compared to controls.

Introduction

Municipal wastewaters and sludges contain a substantial quantity of potentially toxic agents. Hydrocarbons from vehicular traffic (Bomboi and Hernadez 1991), wood preservatives (Wylie et al. 1990), heavy metals (Tanizaki et al. 1992), plus solvents, degreasers, and asbestos may all be discharged to the municipal treatment system. In one study on sewage sludges from 40 plants in England, chromium, copper, lead, manganese and zinc were all above 600 mg/kg dry weight (Sterritt and Lester 1981). The decomposition of organic material also leads to the production of toxic gases, such as H_2S, other breakdown products, such as ammonia-N, and high suspended solid levels.

Although untreated wastewater often induces a wide range of acute and chronic toxic responses in aquatic organisms, the extent of these effects varies with the nature of incoming waste. In some cases, highly toxic industrial chemicals, such as pentachlorophenol, may be discharged to the sewer system (Wylie et al. 1990; Melcer and Bedford 1988). Domestic waste, containing substantial amounts of organic material, is often less toxic, particularly if a large quantity of dilution water accompanies the waste.

At present, a substantial amount is known about the acute toxicity of municipal wastewaters, but there have only been a few studies of the chronic effects of such wastes. Chronic studies are now, however, more realistic considering the widespread implementation of modern in-plant treatment systems. The purpose of this study was to determine the toxicity of wastewater from two municipal treatment plants situated in Edmonton, Alberta.

Chronic effects were determined using rainbow trout (*Oncorhynchus mykiss*), and a cladoceran (*Daphnia magna*). A mollusc (*Anondonta grandis*) and fathead minnow (*Pimephales promelas*) were also used in acute toxicity tests.

Materials and Methods

Municipal Wastewater Treatment Plants

Both plants (Edmonton Capital Region, Edmonton Goldbar) used a mechanical-biological system to treat wastewater. The systems were essentially similar, as outlined below, and did not chlorinate the final effluent.

Capital Region

Screening > grit removal > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (North Saskatchewan River).

Goldbar

Grit removal > bar screens > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (North Saskatchewan River).

Collection Procedures and Sample Manipulation

Acute toxicity samples were collected on 30 November 1989 and 21 June 1990 (Capital Region plant), and 23 November 1989 and 14 June 1990 (Goldbar plant). These sampling dates were selected to parallel cold weather and warm weather operating conditions, respectively. The chronic toxicity procedures involved the collection of an initial sample, followed by three replacement samples. The sampling dates were 16 November, 23 November, 30 November, and 7 December 1990 (Capital Region), and 12 October, 19 October, 26 October, and 2 November, 1990 (Goldbar). All samples were collected, transported, and manipulated as described in Moore et al. (under review).

Toxicological Procedures

All test animals were acquired and maintained for at least one month following Standard Operating Procedures (Aquatic Biology Branch, 1991). A summary of water quality conditions during maintenance is given in Table 1. The acute toxicologic procedures involving rainbow trout, fathead minnow, *Daphnia magna*, and *Anondonta grandis* were conducted using Standard Operating Procedures (Aquatic Biology Branch 1991; Moore et al. under review). Examples of water quality conditions in test chambers during implementation of the above-noted toxicologic procedures are listed in Tables 2a and 2b.

The 21-d chronic procedure using *Daphnia magna* was conducted using effluent from both plants. The method followed Standard Operating Procedures (Aquatic Biology Branch 1991), and is essentially similar to other published methods (USA Environmental Protection Agency 1987). The wastewater was completely replaced on four occasions during the study. Because the influent was often highly turbid, it was not possible to implement the *Daphnia magna* procedure on such samples.

A 28/29-d chronic study involving rainbow trout was used to evaluate potential effects of the effluent on growth. Three groups of 10 rainbow trout each were tested. Exposure concentrations were 100% effluent, 50% effluent, and a control. The tests were conducted in polypropylene pails containing 40 L of medium at a temperature of 15°C. Wet weight and fork length of all fish were measured at the start and end of the experiment. Size of fish at the start of the experiment was relatively homogeneous (see Results). Fish were fed their standard ration of a commercial feed during the experiment. The medium was replaced on four occasions, as outlined preceding. The Capital Region test was conducted for 28 d whereas the Goldbar test was extended an additional day due to work schedule restraints.

Table 1. Summary (average, range) of water quality conditions used during the maintenance of the four test species.

Species	Temp. °C	D.O. mg/L	Cond. µS/cm	pH
O. mykiss	13.0 (10.4-15.0)	10.6 (9-13)	220 (210-270)	7.6 (7.0-8.1)
P. promelas	22.0 (13.8-27.0)	7.1 5.0-8.1)	290 (271-605)	7.9 (7.1-9.4)
D. magna	20.1 (18.6-21.1)	7.1 (4.9-8.3)	546 (324-627)	8.1 (7.4-9.1)
A. grandis	21.0 (17.6-22.9)	7.3 (6.4-9.6)	560 (298-787)	8.1 (7.8-8.3)

Table 2a. Summary (average, range) of water quality conditions in control chambers during implementation of toxicologic procedures.

Procedure	Temp. °C	D.O. mg/L	Cond. µS/cm	pH
O.mykiss	14.8	9.0	300	7.5
96-h LC_{50}	(14.5-15.1)	(8.8-9.3)	(295-307)	(6.3-8.1)
P. promelas	20.4	7.6	307	7.6
96-h LC_{50}	(20.0-21.0)	(6.9-8.2)	(279-319)	(6.5-8.1)
A. grandis	18.5	8.2	315	7.3
96-h LC_{50}	(16.8-21.0)	(7.5-8.8)	(277-345)	(5.8-8.1)
D. magna	20.1	8.0	522	7.7
48-h LC_{50}	(19.9-22.0)	(7.2-8.9)	(334-641)	(7.0-8.1)

Table 2b. Summary (average, range) of water quality conditions in undiluted (100%) wastewater during implementation of toxicologic procedures.

Procedure	Temp. °C	D.O. mg/L	Cond. µS/cm	pH
O. mykiss	15.1	8.6	1171	7.5
96-h LC_{50}	(14.9-17.7)	(7.5-9.0)	(1111-1240)	(5.9-8.1)
P. promelas	20.3	7.5	1165	7.4
96-h LC_{50}	(19.3-21.2)	(4.0-8.1)	(975-1336)	(5.9-8.5)
A. grandis	18.3	8.1	1179	7.0
96-h LC_{50}	(17.2-21.2)	(6.4-8.7)	(975-1393)	(5.3-8.1)
D. magna	20.5	7.7	1117	7.0
48-h LC_{50}	(18.9-21.8)	(5.4-8.4)	(803-1352)	(6.0-8.0)

Results

Capital Region Wastewater Treatment Plant

Chemical Analysis: The majority of metals underwent a reduction in concentration during the treatment process (Table 3). Concentrations of highly toxic metals such as cadmium and copper were low, particularly in the effluent. Ammonia-N in the influent ranged from 32 to 36 mg/L, but also fell sharply in the effluent. At a pH of 7.5, the percent of unionized ammonia-N is 0.86 and 1.24 at temperatures of 15° and 20°C, respectively (Piper et al. 1982). Hence the highest concentration of ammonia-N (36 mg/L) was equivalent to 0.31 and 0.44 mg/L of unionized ammonia-N at these respective temperatures.

Table 3. Chemical analysis (mg/L) of some important water quality parameters in municipal wastewater collected from the Capital Region plant.

Date	30 November 1989		21 June 1990	
Parameter	Influent	Effluent	Influent	Effluent
Aluminum	0.488	0.074	0.393	0.218
Arsenic	0.0015	0.0011	0.0014	0.0014
Cadmium	0.002	0.001	0.003	0.003
Chromium	0.037	0.027	0.008	0.012
Copper	0.23	0.002	0.026	0.011
Lead	0.022	0.003	0.006	0.010
Zinc	0.093	0.066	0.062	0.047
Ammonia-N	35.9	0.05	32.0	6.40

Acute Toxicity and Histopathologic Evaluation: Influent and effluent were non-acutely toxic to the four test species. LC_{50}s were always ≥100%, regardless of when the samples were collected. No histopathologic abnormalities were noted in any tissue of rainbow trout and fathead minnow exposed to influent and effluent collected on 21 June 1990. When rainbow trout were exposed to influent collected on 30 November 1989, blunting and edema of gills were observed in all fish at 100% concentration. Although the skin of this species also showed epithelia hyperplasia, no other anomalies were noted.

Chronic Toxicity: There was a statistically significant increase ($p<0.05$) in the production of neonates of *Daphnia magna* at all effluent concentrations compared to controls (Table 4). Hence, the No-Observed-Effect-Concentration and the Lowest-Observed-Effect-Concentration could not be calculated. Likewise, mortality was significantly less ($p<0.05$) at all effluent concentrations except 100% compared to controls (Table 4).

Table 4. Mortality and production of neonates by *Daphnia magna* exposed for 21 d to different concentrations of effluent from the Capital Region treatment plant.

Parameter	Effluent Concentration Control	6%	12%	25%	50%	100%
Sample Size	10	10	10 10	10	10	
Mortality	7	2*	1* 0*	0*	3	
Neonates/adult (average)	5.1	32.9	42.4	48.0	68.7	45.5

Notes: *Different from control at $p<0.05$.
Number of neonates/adult significantly greater than control at all effluent concentrations ($p<0.05$).

There was no dose-related or statistically significant ($p>0.05$) pattern of mortality when rainbow trout were exposed to effluent concentrations of 100% and 50% for 28 d (Table 5). Similarly fish body weight, fish length, and body weight gains were not significantly different ($p>0.05$) from control fish (Table 5).

Table 5. Mortality, wet weight and fork length of rainbow trout exposed for 28 d to different concentrations of effluent from the Capital Region treatment plant.

Parameter	Control	50% Effluent	100% Effluent
Mortality	0	1	1
Wet weight (g)	1.80±0.19	1.87±3.5	1.79±0.54
Fork length (cm)	5.6±0.3	5.6±0.3	5.8±0.54
Weight gain (g)	0.59±0.19	0.66±0.35	0.58±0.54
Sample size at 28 d	10	9	9

Notes: Initial mean body weight (g) based on 35 fish, 1.21±0.09 g
Each group contained 10 rainbow trout at the start of the experiment
Mortality data are not significantly different ($p>0.05$)
Weight data are not significantly different ($p>0.05$).

Goldbar Wastewater Treatment Plant

Chemical Analysis: The majority of metals underwent a reduction in concentration during the treatment process (Table 6). The concentrations of highly toxic metals such as cadmium and copper were low, particularly in the effluent. Chromium residues increased from 0.135 to 0.186 mg/L in the June 14, 1990 samples. It is not known if this increase represents a sampling artifact or an actual increase in concentration. Ammonia-N was recorded at 29-45 mg/L in the influent and showed only a modest decline in the effluent. At a pH of 7.5, the percent of unionized ammonia-N is 0.86 and 1.24 at temperatures of 15° and 20°C, respectively (piper et al., 1982). Hence the highest concentration of ammonia-N (45 mg/L) was equivalent to 0.39 and 0.56 mg/L of unionized ammonia-N at these respective temperatures.

Acute Toxicity and Histopathologic Evaluation: Effluent was non-acutely toxic to the four test species. LC$_{50}$'s were always $\geq$ 100%, regardless of when the samples were collected. The influent was also non-acutely toxic (LC$_{50}$$\geq$100%) except for the November 23, 1989 sample, which yielded an LC$_{50}$ of 95.6% for fathead minnow.

Table 6. Chemical analysis (mg/L) of some important water quality parameters in municipal wastewater collected from the Goldbar plant.

Date	23 November 1989		14 June 1990	
Parameter	Influent	Effluent	Influent	Effluent
Aluminum	0.117	0.014	0.930	0.019
Arsenic	0.0009	0.0006	0.0027	0.0008
Cadmium	0.005	0.002	0.006	0.003
Chromium	0.119	0.008	0.135	0.186
Copper	0.028	0.002	0.043	0.004
Lead	0.016	0.011	0.052	<0.002
Zinc	0.096	0.078	0.143	0.039
Ammonia-N	45.0	19.0	28.9	20.6

Rainbow trout developed mild to moderate hyperplasia of the epithelial cells of the skin when exposed to influent (60-100% concentrations) from the November and June collections. This condition was not observed in controls. The trout also exhibited diffuse mild blunting, atrophy and edema of the gill lamella when exposed to influent. Since this lesion was also found in the controls, it was probably not due to exposure to wastewater. No lesions were found in fathead minnow exposed to the influent. In addition no lesions were found in rainbow trout and fathead minnow exposed to effluent collected on both collection dates from the plant.

Chronic Toxicity: There was a statistically significant increase ($p < 0.05$) in the production of neonates of *Daphnia magna* at all effluent concentrations compared to controls (Table 7). Hence the No-Observed-Effect-Concentration and the Lowest-Observed-Effect-Concentration could not be calculated. Mortality of *Daphnia magna* in 25% and 100% effluent concentrations was significantly less ($p<0.05$) than controls (Table 7). At other concentrations there was no significant difference ($p>0.05$) in mortality (Table 7).

There was no dose-related or statistically significant pattern of mortality when rainbow trout were exposed to effluent concentrations of 100% and 50% for 29 d (Table 8). Similarly fish body weight, fish length, and body weight gains were not significantly different from control fish (Table 8).

Table 7. Mortality and production of neonates by *Daphnia magna* exposed for 21 d to different concentrations of effluent from the Goldbar treatment plant.

		Effluent Concentration				
Parameter	Control	6%	12%	25%	50%	100%
Sample size	10	10	10	10	8	10
Mortality	4	1	1	0*	1	0*
Neonates/adult (average)	26.7	56.6	77.7	69.8	75.9	110.9

Notes: *Different from controls at $p<0.05$.
Number of neonates/adult significantly greater than control at all effluent concentrations ($p<0.05$).

Table 8. Mortality, wet weight and fork length of rainbow trout exposed for 29 d to different concentrations of effluent from the Goldbar treatment plant.

Parameter	Control	50% Effluent	100% Effluent
Mortality	1	1	3
Wet weight (g)	5.15±0.74	5.73±0.86	4.77±.47
Fork length (cm)	7.5±0.3	7.9±0.4	7.3±0.7
Weight gain (g)	1.87±0.73	2.45±0.85	1.49±1.47
Sample size at 28 d	9	9	7

Notes: Initial body weight (g) based on 33 fish, mean weight = 3.28±0.2 g. Each group contained 10 rainbow trout at the start of the experiment. Mortality data are not significantly different ($p<0.05$). Weight data are not significantly different ($p<0.05$).

Discussion

The increase in neonate production and low mortality of *Daphnia magna* during the 21-d exposure period was probably due to improvement in the quantity and/or quality of food in the organically rich samples. Enhanced nutritional conditions are widely known to improve the physical condition of aquatic animals, and possibly improve their ability to tolerate potentially toxic environments. There may have been potentially toxic agents in the effluents of this study, but their effects were apparently masked by improved nutritional conditions. Such interactions can be considered antagonistic in nature, and the same response might be expected with other organically rich effluents, such as those from pulp and paper mills. During our sampling trips to lagoon systems (Camrose, Fort McMurray, Wetaskiwin) in a related study (Moore et al., under review), we were able to observe and collect large numbers of *Daphnia magna* from the sewage lagoons.

Seven-day exposure of the cladoceran *Ceriodaphnia dubia* to effluent from a plant in Galt (Ontario) resulted in impairment of reproduction at 39-71% effluent (Ontario Ministry of Environment 1990). Three seven-day chronic tests of lethality were also conducted on the same effluent. The LC_{50}s ranged from 46 to 88%, and one sample was not lethal. These effects were likely due to chlorination of the final effluent and presence of ammonia-N at relatively high concentrations. Neiheisel et al. (1988) also used the seven-day survival and reproduction test with *Ceriodaphnia dubia* on effluents from several treatment plants in Ohio. The No-Observed Effect-Concentration in both aspects of the test generally ranged from 3 to 30%. Toxic wastewater was produced when specific industrial chemicals, which could not be efficiently treated by the plants, were discharged to the influent.

Our chronic rainbow trout test resulted in no growth over the 28/29 d duration of the procedure, despite the fact that the fish were regularly fed. The lack of growth may have resulted from at least one of the following factors: i) presence of toxic agents; ii) stress of confinement to relatively small (40 L) test chambers; and iii) handling stress. The relative significance of these factors is not known, but the lack of increased mortality over the length of the test suggests that the presence of toxic agents had only a mild effect on the fish. Unlike *Daphnia magna*, rainbow trout could not use for food the finely divided organic material associated with the effluent. Wylie et al. (1990), using a seven-day larval fathead minnow test to investigate toxicity of municipal effluents in southwest Missouri, found that the minnows died within a few hours of exposure in all but the most highly diluted samples. This was due to incomplete removal of pentachlorophenol from the system.

The wastewaters used in this study were generally non-acutely toxic to the four test species, and there appeared to be little difference in the toxicity of the influent and effluent samples. Although rainbow trout developed lesions associated with the epithelial cells of the skin following exposure to influent, these disorders were relatively minor and did not affect the survival of fish. Furthermore, fathead minnow did not develop any lesions. By contrast, Narain et al. (1990) noted enormous damage to the gills of the perch *Anabas testudieneus* exposed to untreated sewage from a city in India. The lesions included lamellar fusion, separation of gill epithelium from supposing cells, stasis of lamellar blood circulation, and rupture of gill epithelium. Narain and Srivastava (1989) similarly showed that ammonia-N in sewage was the primary agent in the development of major haematological disorders in the catfish *Heteropneustes fossilis*.

In summary, when ammonia-N was reduced, wastewater from the two plants was usually non-acutely toxic to four test species, and induced in fish only minor disorders associated with the skin. Long term exposure of *Daphnia magna* to the effluents resulted in an increase in production of neonates. Similarly there was no change in mortality or gains in weight and length when rainbow trout were exposed to the effluents for 28/29 d.

Acknowledgements

This work would not have been possible without the assistance of plant managers, who not only provided information concerning their plants but also arranged for plant tours and assistance in collecting samples. The plant managers who assisted in this study were: Bryan Hystad (Edmonton-Capital Region) and Glenn Brown (Edmonton-Goldbar). This study was initiated at the request of Municipal Branch (Standard and Approvals Division, Alberta Environment). Municipal Branch staff assisted in the development of the study design, made initial contact regarding the study with plant managers, arranged for chemical analysis of wastewater samples, and reviewed the final report. Dr. F. Skinner, Environmental Technology Division (Alberta Environmental Centre), provided useful advice on sample manipulation and ammonia removal from samples. Analysis of heavy metals in all samples was conducted by Water Analysis Branch (Alberta Environmental Centre).

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CANADIAN NETWORK OF TOXICOLOGY CENTRES: NEW DIRECTIONS IN TOXICOLOGY. Keith R. Solomon, Canadian Network of Toxicology Centres, Centre for Toxicology, University of Guelph, 645 Gordon St., Guelph, ON.

The Canadian Network of Toxicology Centres (CNTC) was formed in 1988 in a Memorandum of Understanding by the Centre interuniversitaire de recherche en toxicologie, Université de Montréal and Université de Québec à Montréal; the Centre for Toxicology, University of Guelph; and the Toxicology Research Centre, University of Saskatchewan. In 1992, the federal government established a National Toxicology Network at universities. The Network is operated by the CNTC and will work with universities and other partners to establish toxicology research centres across Canada so that resources and information can be shared.

As envisaged in the GREEN PLAN, the Network will provide relevant, timely and credible toxicological data, advice and judgment on toxicological issues of significance to Canada. The core funding provided under the GREEN PLAN will support and maintain a program of multidisciplinary toxicological research. The Network will allow the formation of a critical mass of expertise in key sub-specializations of toxicology in Canada.

This presentation will summarize developments to date and describe the research planning process to attain these objectives.

EVALUATION OF A UNIQUE ECOSYSTEM IN SOUTHEASTERN WASHINGTON, USA: THE HANFORD SITE.

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ABSTRACT

Environmental monitoring has been conducted on the USA Department of Energy's Hanford Site for almost 50 years to evaluate potential impacts of nuclear operations on air, surface and ground water, foodstuffs, fish, wildlife, soils and vegetation. In recent years, measured Hanford Site perimeter concentrations of airborne radionuclides, and concentrations of radionuclides and non-radiological water quality in the Columbia River have been in compliance with applicable guidelines or standards. Foodstuffs irrigated with river water downstream of the Site had radionuclide levels similar to those in foodstuffs from control areas. Radionuclide levels in onsite wildlife samples and soils and vegetation from on- and offsite locations were typical of those attributable to worldwide fallout. The doses to a maximally exposed individual using worst-case assumptions for all routes of exposure have ranged from 0.03 to 0.09 mrem/yr from 1985-1990. Chinook salmon spawning in the Columbia River at Hanford has generally increased with a concomitant increase in winter roosting activity of bald eagles. An elk herd, established by immigration in 1972, is also increasing. The Site currently serves as a refuge for Canada goose, great blue heron, and various plants and other animals, e.g., mule deer and coyote. These and other attributes of the Hanford ecosystem will be discussed.

INTRODUCTION

The USA Department of Energy's (DOE) Hanford Site occupies a land area of about 1,450 km^2 (560 mi^2) in semi-arid southeastern Washington, USA (Figure 1). The Columbia River flows through the Site and forms part of its eastern boundary. River flow is regulated daily according to electric power demands. Average river flow (68-yr average) is 120,000 cfs (McGavok et al. 1987). The southwestern portion of the Site includes the southern terminus of the Rattlesnake Hills with elevations exceeding 1000 m. Both confined and unconfined aquifers lie beneath the Site.

Nuclear and non-nuclear industrial and research activities have been conducted at Hanford since 1943. The most environmentally significant activities have involved the production of nuclear materials, and the chemical processing and waste management associated with the major product, plutonium. By-product wastes have included gamma, beta, and alpha-emitting radionuclides and various nonradioactive chemicals in gaseous, liquid and solid forms.

There are four major operations areas on the Hanford Site (Figure 1). The 100 Areas located along the Columbia River include the dual-purpose N Reactor (now deactivated) that produced plutonium for national defense and steam for the Hanford Generating Project (HGP), operated by the Washington Public Power Supply System (WPPSS), and eight deactivated, single-purpose, plutonium production reactors. The plutonium uranium extraction (PUREX) plant (reactor fuel reprocessing), plutonium finishing plant (Z Plant), and waste-disposal facilities are located in the 200 Areas on a plateau (elevation 229 m) about 11.3 km west of the Columbia River. The 300 Area, located just north of Richland, Washington contains the uranium fuel manufacturing facilities that supported N Reactor, the single purpose reactors, and several research and development laboratories. The Fast Flux Test Facility (FFTF) which has operated intermittently since 1981 to test new fuels and materials for future breeder reactor technology and the WPPSS Nuclear Plant (WNP) sites are located in the 400 Area. Non-government facilities within Hanford Site boundaries include HGP, WNP Sites-1, 2 and 4, and a commercial low-level radioactive-waste burial site, operated by USA Ecology, near the 200 Areas. WNP-2 is a commercial reactor that achieved full operation status in the fall of 1984. The Siemens Nuclear Power Corporation fuel fabrication facility (formerly Advanced Nuclear Fuels Corp., formerly Exxon) is adjacent to, but not located on, Hanford Site property.

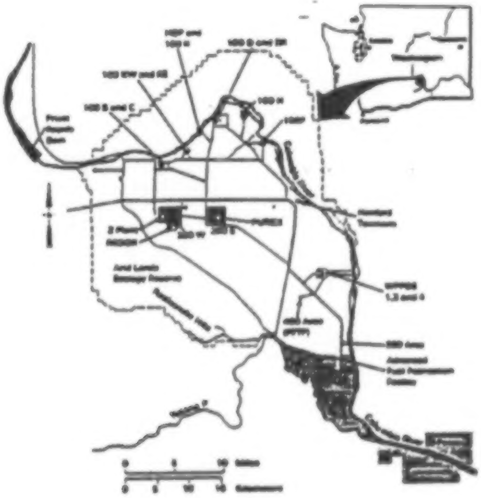

Figure 1. The Hanford Site, B, C, KW, KE, D, DR, N, H, F = production reactors; HGP = Hanford Generating Project; REDOX = reduction-oxidation; PUREX = plutonium uranium extraction; WPPSS = Washington Public Power Supply System; FFTF = Fast Flux Test Facility

Environmental monitoring has been conducted at Hanford almost 50 years to assess potential impacts to individuals and populations that may be exposed to radionuclides, ionizing radiation and hazardous chemicals. Environmental media sampled include air, surface and ground waters, foodstuffs (fruits, vegetables, milk, etc.), fish, wildlife, soils and vegetation. Fish and wildlife are monitored for radioactivity and to determine the population status of key species. Recently, three community-operated, environmental surveillance stations were constructed and began operations in towns near the Hanford Site (Gray and Brown 1991, Woodruff and Hanf 1992). Citizens, usually a local high school science teacher and an alternate, operate the stations. Results are compared to, and reported with, those obtained from the larger environmental monitoring network.

In 1967, the Arid Lands Ecology Reserve was established on the northeastern slope of Rattlesnake Mountain (Figure 1) as an outdoor laboratory for environmental research in the shrub-steppe (Rickard et al. 1988). In 1977, the Site was dedicated as a National Environmental Research Park. In 1992, DOE signed a cooperative agreement with The Nature Conservancy to characterize, preserve, and protect rare biotic species and communities. These activities could not be conducted elsewhere because of human intrusion.

RADIOLOGICAL MONITORING

Air

Potential airborne transport of stack releases containing radionuclides from Hanford facilities offers a direct pathway for human exposure. Air is sampled continuously for airborne particulates and analyzed for radionuclides at 50 onsite and offsite locations, including 14 in nearby and distant communities, three of which were operated by the public (Woodruff and Hanf 1992). At selected locations, gases and vapors are also collected and analyzed. Many of the longer-lived radionuclides released at Hanford are also present in atmospheric fallout that resulted from nuclear weapons testing in the 1950s and 1960s or from nuclear accidents that occurred elsewhere.

In May and June, 1986, air samples collected onsite as well as those from distant locations showed increases in several long- and short-lived radionuclides (e.g., ^{137}Cs, ^{131}I, ^{103}Ru) that resulted from the reactor accident at Chernobyl, April, 1986, in western Russia. However, even then, no sample exceeded 0.17% of the applicable DOE derived concentration guide (DCG) for areas permanently occupied by the public (PNL 1986).

Highest doses to people from past radiological releases to air, occurred from 1944-1947. Iodine-131, a short-lived radionuclide, accounted for more than 80% of the dose. People could have been exposed to radionuclides in air through inhalation, consumption of food crops and drinking milk from cows pastured downwind of Hanford. Actual radiological doses depend on location, life style, age and other factors. Preliminary estimates suggest that the most exposed group, infants and small children who drank milk from cows pastured downwind of Hanford during 1944-1947, may have received 15 to 650 rad to the thyroid (PNL 1990a).

Surface Water

Columbia River water is used for drinking at downstream cities, and for crop irrigation and recreational activities (fishing, hunting, boating, waterskiing, swimming). Thus, it constitutes the primary environmental pathway to people for radioactivity in liquid effluents. Radionuclides can be delivered to people through crops irrigated with river water and cow's milk through irrigated alfalfa and other cattle forage. Although radionuclides associated with Hanford operations, worldwide fallout and natural phenomena continue to be found in small but measurable quantities in the Columbia River, concentrations are below Washington State and Environmental Protection Agency (EPA) Drinking Water Standards (DWS).

Deep sediments in downstream reservoirs still contain low concentrations of some long-lived radionuclides (Nelson and Haushild 1970, Haushild et al. 1975, Robertson and Fix 1977, Sula 1980, Beasley et al. 1981). Trace amounts of ^{239}Pu, ^{60}Co, ^{137}Cs, and ^{152}Eu persist in sediments accumulated above the first downstream dam (McNary). In 1977, about 20 to 25% of the total plutonium inventory (^{239,240,241}Pu) in Lake Wallula sediments, 100 km downstream, was believed to originate from the 1944 through 1971 releases at Hanford (Beasley et al. 1981). However, only ^{239}Pu was believed to actually reflect earlier reactor operations. Further, this ^{239}Pu was derived from ^{239}Np (produced by neutron capture in natural uranium followed by decay to ^{239}Np), an abundant isotope in Columbia River water. Thus, plutonium may not have actually been released to the river from reactor operations.

Highest doses to people from past radiological releases to the Columbia River probably occurred from 1964 to 1966. People could have been exposed to radionuclides directly through swimming, drinking river water or eating Columbia River fish. Thus, radiological doses depend on location, life style, and other factors. Preliminary estimates suggest these doses may have ranged from 5 to 180 mrem for people living in the Tri-City area (Richland, Kennewick, Pasco) during that time period (PNL 1990b).

Groundwater

The shallow unconfined aquifer has been affected by waste-water disposal practices at Hanford more than the deeper, confined aquifers. Discharge of water from various industrial processes has created ground-water mounds near each of the major waste-water disposal facilities in the 200 Areas, and in the 100 and 300 Areas (Figure 1). Discharge to groundwater in the 200 Areas may have contributed ten times more water annually to the unconfined aquifer than natural input from precipitation and irrigation (Graham et al. 1981). These groundwater mounds have altered local flow patterns in the aquifer, which are generally from west to east.

Groundwater, primarily from the unconfined aquifer, is currently sampled from over 450 wells and analyzed for radionuclides (Woodruff and Hanf 1992). Tritium (^{3}H) occurs at relatively high levels in the unconfined aquifer, is one of the most mobile radionuclides, and thus, reflects the extent of groundwater contamination from onsite operations. Many liquid wastes discharged to the ground at Hanford have contained ^{3}H. Tritium from releases prior to 1983 that passed-downward through the vadose (unsaturated) zone to the unconfined aquifer continues to move with groundwater flow toward the Columbia River. Tritium concentrations in Hanford groundwater range from less than 300 pCi/L to over 2,000,000 pCi/L near or within the 200 Areas (PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, Woodruff and Hanf 1991, 1992).

Groundwater from the unconfined aquifer enters the river through subsurface flow and springs that emanate from the riverbank. McCormack and Carlile (1984) identified 115 springs along a 41-mile stretch of river. Tritium concentrations in wells near the springs ranged from 19,000 to 250,000 pCi/L and averaged 176,000 pCi/L in 1985 (Price 1986). Although the distribution of ^{3}H and other radionuclide concentrations in springs generally reflected those in nearby ground-water wells, the magnitude was generally less in springs due to mixing of ground and surface water. Tritium concentrations in the river were generally less than those in springs. Tritium concentrations in springs were less than 4% of the DOE DCG (2,000,000 pCi/L). Tritium concentrations in the river were less than 0.5% of the DCG and less than half the regulatory limit for drinking water (20,000 pCi/L) (EPA 1976). It is noteworthy that ^{3}H also occurs naturally in the Columbia River upstream of Hanford. From 1983-1989, annual average ^{3}H concentrations in the river (<200 pCi/L) were at least a factor of 100 below the drinking water limit (Jaquish and Bryce 1989, 1990).

Dirkes (1990) sampled the springs in a follow-up study and reported similar results. Except for ^{90}Sr near N-reactor, radionuclide concentrations (including ^{3}H) in springs were below the DCG. However, ^{3}H concentrations were above the EPA DWS in some springs. Non-radiological constituents were generally undetectable in springs. Concentrations of (radiological and non-radiological) constituents in the river were below the DWS immediately downstream of the mixing zone. The only radiological constituents found above detection limits in the river were ^{3}H, ^{99}Tc, and ^{234,238}U, although ^{90}Sr, ^{129}I and, ^{235}U and ^{239,240}Pu have been reported at low but detectable levels by Jaquish and Bryce (1990).

Discharges to the river across from Hanford (seeps and irrigation returns) showed higher gross alpha, gross beta and ^{234,235,238}U than in the river. Uranium is found in Franklin County groundwater, the Spokane River drainage system and may also reflect use of phosphate fertilizers.

Foodstuffs

The most direct way for deposited radionuclides to enter the human foodchain is through consumption of leafy vegetables. Samples of alfalfa and several foodstuffs, including milk, vegetables, fruit, beef, chickens, eggs and wheat, are collected from several locations, primarily downwind (i.e., south and east) of the Site (Price 1986, PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, 1990, Woodruff and Hanf 1991, 1992). Samples are also collected from upwind and somewhat distant locations to provide information on radiation levels attributable to worldwide fallout. Foodstuffs from the Riverview Area (across the river and southeast) are irrigated with Columbia River water withdrawn downstream of the Site. Although low levels ^{3}H, ^{90}Sr, ^{129}I, and ^{137}Cs have been found in some foodstuffs, concentrations in samples collected near Hanford are similar to those in samples collected away from the Site.

Highest doses to people from past radiological releases that entered the food chain involved consumption of milk by infants from 1944-1947 (PNL 1990a) as discussed above.

Fish and Wildlife

Fish are collected at various locations along the Columbia River and boneless fillets are analyzed for ^{60}CO, ^{90}Sr, and ^{137}Cs. Carcasses are analyzed to estimate ^{90}Sr in bone. Following shutdown of the last once-through cooling reactor and installation of improved liquid effluent control systems at N Reactor, short-lived radionuclides, including the biologically important ^{32}P and ^{65}Zn, essentially disappeared from the river (Cushing et al. 1981) through radioactive decay. Radionuclide concentrations in fish collected from the Hanford Reach of the Columbia River are similar to those in fish from upstream locations.

Deer (*Odocoileus* sp.), ring-necked pheasants (*Phasianus colchicus*) mallard ducks (*Anas platyrhynchus*), Nuttall cottontail rabbits (*Sylvilagus nuttallii*) and black-tailed jack rabbits (*Lepus californicus*) are collected and tissues are analyzed for ^{60}Co and ^{137}CS (Muscle) ^{239,240}Pu (liver) and ^{90}Sr (bone). The doses that could be received by consuming wildlife at the maximum radionuclide concentrations measured in 1985-1991 were below applicable DOE standards (Price 1986, PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, 1990, Woodruff and Hanf 1991, 1992).

Soils and Vegetation

Airborne radionuclides are eventually deposited on vegetation or soil. Samples of surface soil and rangeland vegetation (sagebrush) are currently collected at 23 onsite and 29 site perimeter and offsite locations (Woodruff and Hanf 1992). Samples are collected from nonagricultural, undisturbed sites so that natural deposition and buildup processes are represented. Sampling and analyses in 1985 through 1989 showed no radionuclide buildup offsite that could be attributed to Hanford operations (Price 1986, PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, 1990, Woodruff and Hanf 1991, 1992).

Penetrating Radiation

Penetrating radiation (primarily gamma-rays) is measured in the Hanford environs with thermoluminescent dosimeters to estimate dose rates from extremal radiation sources. Radiation surveys are routinely conducted at numerous onsite locations including roads, railroads and retired waste-disposal sites outside of operating areas. Onsite and offsite measurements and survey results for 1985-1991 were similar and comparable to past years. Dose rates near some operating facilities were only slightly higher than natural background rates.

Overall Radiological Impact from Hanford Operations

Reconstruction of past (1940s through 1960s) radiological doses to people living in communities surrounding Hanford is currently under way. Preliminary estimates for certain pathways are available (PNL 1990a, 1990b), as discussed above. Although more refined estimates are unavailable, these studies are continuing.

Beginning in 1974, the evaluation of radiation doses has included assessment of the maximum extremal dose rate at a location accessible to the general public, doses to a hypothetical maximally exposed individual, and doses to the population within 80 km (50 miles) of the Site. The calculated 50-year wholebody cumulative dose received by the maximally exposed individual ranged from 0.5 to 3 mrem during the years 1981 through 1986 (PNL 1987). The maximally exposed individual is a hypothetical person who receives the maximum calculated radiation dose when worst-case assumptions are used concerning location, inhalation of radioactive emissions, consumption of contaminated food and water, and direct exposure to contaminants. Expressed as effective dose equivalents, the calculated dose received by a hypothetical maximally exposed individual was 0.02 to 0.1 mrem annually from 1985 through 1991. The average per capita effective dose for 1985 through 1991, based on the population of 330,000 to 340,000 people living within 80 km of the Site, was <0.01 to 0.03 mrem annually (Price 1986, PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, 1990, Woodruff and Hanf 1991, 1992). These estimates and the measured Hanford area background radiation can be compared to other routinely encountered sources of radiation, such as natural terrestrial and cosmic radiation, medical treatment and x-rays, natural internal body radioactivity, worldwide fallout, and consumer products (Figure 2). Radiation doses to the public from Hanford operations have been consistently below applicable standards, and substantially less than doses from other routinely encountered sources of radiation.

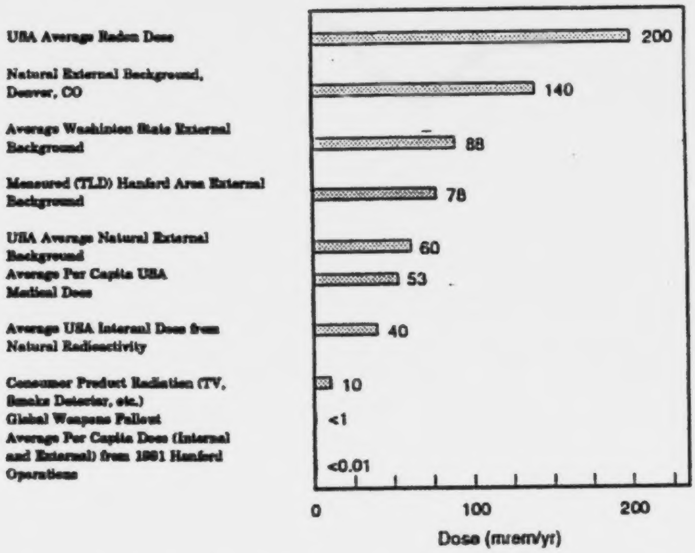

Figure 2. Annual radiation doses from various sources: USA average radon, external background, medical and internal doses, consumer product radiation and weapons fallout from NRCP (1987a); external background, Denver, Colorado from NRCP (1987b). Washington State from Oakley (1972); Hanford external background and average per capita dose from Jaquish and Bryce (1990); TLD = thermo-luminescent dosimeter, does not include neutron component; mrem/yr = millirem per year.

CHEMICAL MONITORING

Air Quality

Nitrogen oxides (NO_x) from fossil-fueled steam and chemical processing facilities, most notably the PUREX plant were sampled by the Hanford Environmental Health Foundation (HEHF) until PUREX operations ceased in 1990. Nitrogen dioxide concentrations measured in 1984-1990 were well below federal (EPA) and local (Washington State) ambient air quality standards (Price 1986, PNL 1987, Jaquish and Mitchell 1988, Jaquish and Bryce 1989, 1990, Woodruff and Hanf 1991).

Columbia River

Nonradioactive waste water is discharged at eight locations along the Hanford reach of the Columbia River. Discharges consist of backwash from water intake screens, cooling water, water storage tank overflow, a building drain, and fish-laboratory waste water. Effluents from each outfall are monitored under a National Pollutant Discharge Elimination System Permit. The Columbia River is also monitored by the United States Geological Survey, upstream and downstream of the Site, to verify compliance with Class A (WSDOE 1977) water-quality requirements.

Numerous studies have evaluated and resolved the potential environmental issues associated with water intake and thermal discharge structures on the Columbia River at Hanford. For example, retrofitting of the HGP water intake and a newer design for the intake used at WNP-2 have ensured safe downstream migration of juvenile chinook salmon (Gray et al. 1979, 1986). Other studies have concluded that thermal discharges from N reactor and HGP to the Columbia River were biologically insignificant (WPPSS 1978, DOE 1982, Neitzel et al. 1982).

GroundWater

In 1991, samples were collected from over 200 groundwater wells and analyzed for chemical constituents. In addition, onsite drinking water sources (not public) were sampled and analyzed by HEHF for water quality. Detected constituents included several metals, anions, coliform bacteria, radionuclides and total organic carbon. Many of these constituents are expected in natural groundwater. Chromium, cyanide, fluoride and carbon tetrachloride were found in wells not used for drinking water near operating areas.

HANFORD FLORA AND FAUNA

Most of the Hanford Site consists of undeveloped land that supports stands of native vegetation and a few exotic species, is free from agricultural practices, and has been essentially free from livestock grazing and hunting for 50 years. Restricted land use has favored native wildlife that frequent riverine habitats, for example, mule deer (*Odocoileus hemionus*), Canada goose (*Branta canadensis*), and great blue heron (*Ardea herodias*). The Site also serves as a refuge for other migratory waterfowl, elk (*Cervus elaphus*), coyote (*Canis latrans*) and a variety of other plants and animals (Gray and Rickard 1989, Fitzner and Gray 1991).

The Columbia River at Hanford supports up to 48 species of fish (Gray and Dauble 1977) and serves as a migration route for upriver runs of Chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*) and sockeye (*O. nerka*) salmon, and steelhead trout (*O. mykyss* formerly *Salmo gairdneri*). The Hanford Reach supports the last remaining mainstem spawning habitat for fall chinook salmon. Steelhead trout also spawn in the Hanford Reach.

Based on redd (nest) counts from the air, fall chinook salmon spawning in the Hanford Reach of the mainstem Columbia River increased dramatically from 1980 through 1989 (Figure 3). Underwater observations by divers (Swan et al. 1988) showed salmon redds at depths below those visible by boat or aircraft suggesting that salmon spawning in the Hanford Reach may be even greater than previously estimated. The increase in salmon spawning has attracted increasing numbers of wintering bald eagles (*Haliaeetus leucocephalus*). The eagle population fluctuates with the salmon population (Figure 3). The bald eagle is listed by the USA Fish and Wildlife Service as "threatened" in the State of Washington (Rickard and Watson 1985).

The sparsely vegetated islands in the Columbia River have historically been used as nesting habitat for Canada goose (Hanson and Eberhardt 1971, Fitzner and Rickard 1982). From the mid-1950s to the mid-1970s the number of goose nests declined from a high of 250-300 to about 100 annually. From the late 1970s to the present, the number of nests has gradually increased to over 300 (Figure 4). Initially, closure of the Hanford Reach was beneficial to the geese by providing freedom from human intrusion.

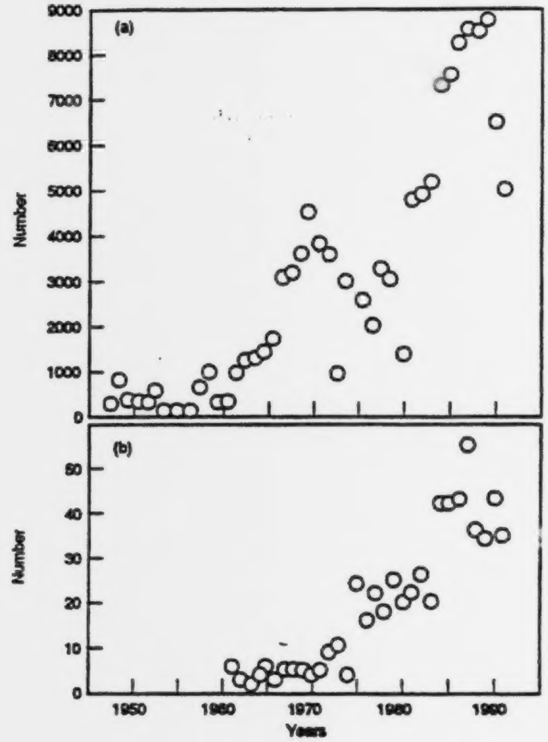

Figure 3. Numbers of (a) Chinook Salmon (*Oncorhynchus tshawytscha*) redds (nests) in the Hanford Reach of the Columbia River, 1947-1991, and (b) Wintering Bald Eagles on the Hanford Site, 1961-1991 (Gray and Rickard 1991, Woodruff and Hanf 1992).

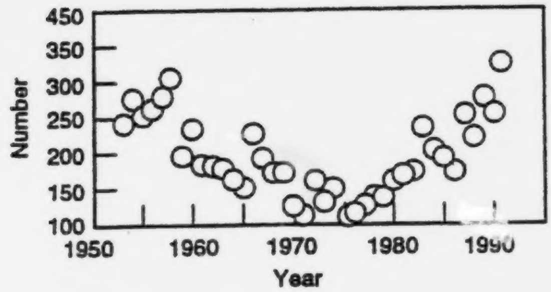

Figure 4. Numbers of active Canada Goose (*Branta canadensis*) nests at Hanford, 1953-1991 (Gray and Rickard 1991, Woodruff and Hanf 1992).

However, the coyote, a natural goose predator, also benefitted, and is believed to have caused the decline in numbers of goose nests into the mid-1970s.

Initially, there were no nesting great blue heron on the Hanford Site. However, there are now four active colonies consisting of about 35-40 or more birds each, and herons are present year round (Gray and Rickard 1989).

Elk first arrived on the Hanford Site in 1972 (Rickard et al. 1977). From a small founding population, the herd size grew to about 80 animals in 1987 (Gray and Rickard 1989) and now exceeds 135 animals (Woodruff and Hanf 1992) (Figure 5). The rapid increase in elk is attributed to the lack of predation or human disturbance during calving, absence of onsite hunting, and the lack of competition from sheep and cattle for available forage. The mule deer population at Hanford is estimated at several hundred animals and appears stable even in the absence of onsite hunting. Coyote predation on fawns is believed to be an important factor that maintains the stable deer population (Steigers and Flanders 1980).

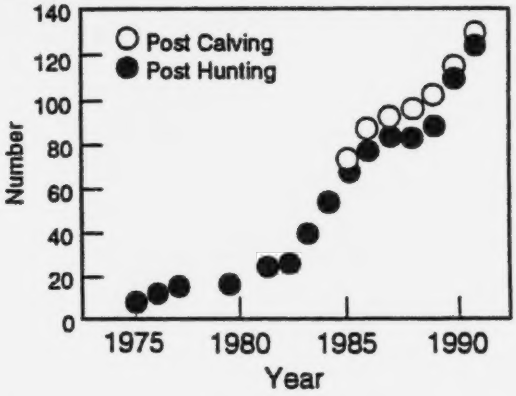

Figure 5. Numbers of elk at Hanford after calving in spring and offsite hunting in the fall, 1975-1991. Special permit hunting offsite began in 1986 (Gray and Rickard 1991, Woodruff and Hanf 1992).

SUMMARY

Environmental monitoring is conducted at the USA DOE Hanford Site to assess potential effects of Hanford Operations on the local environs, onsite workers, and the offsite public. Monitoring for radiological emissions has been ongoing for almost 50 years and includes air, surface and ground waters, foodstuffs, fish, wildlife, soil and vegetation. Measured and calculated radiation doses to the public have been consistently below applicable regulatory limits. Monitoring of fish and wildlife is a significant component of the program. The Hanford Site now serves as a refuge for key fish and wildlife species.

ACKNOWLEDGMENTS

Environmental monitoring at Hanford reflects the cooperative efforts of numerous individuals representing the staffs of DOE, PNL, HEHF, and other contractor, state and federal organizations. Environmental monitoring has been conducted by PNL since 1965, and is supported by DOE under Contract DE-AC06-76RLO 1830 with Battelle Memorial Institute.

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EFFECTS OF A MOLT-ACCELERATING INSECTICIDE ON STREAM INVERTEBRATES.

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A new molt-accelerating insecticide (RH5992) is being evaluated for potential use in forestry to control defoliating lepidopterans. This compound is a nonsteroidal ecdysone agonist that mimics the molting hormone 20-hydroxyecdysone in larval insects and exhibits insecticidal activity by inducing premature and incomplete molting of larvae. The potential adverse effects of RH5992 on stream invertebrates were evaluated in a two-tiered test system. Acute lethal effects were determined for one amphipod and 11 species of aquatic insects in laboratory flow-through bioassays. Lethal and behaviourial effects (drift response) on the amphipod and 8 species of stream insects were then evaluated under natural environmental conditions and more realistic exposure regimes in outdoor stream channels. In addition, an experiment to determine effects of RH5992-contaminated foliage on the growth and survival of shredding macroinvertebrates was also conducted in the stream channels. Results from these experiments will be presented, and the environmental hazard and further developments of this new product will be discussed.

TOXICITY OF CYANOBACTERIAL BLOOMS IN ALBERTA LAKES. B.G. Kotak*[1], S.E. Hrudey*[2], S.L. Kenefick*[2] and E.E. Prepas*[1], Department of Zoology, University of Alberta, Edmonton, AB, T6G 2E9, *[2] Environmental Health Program, Faculty of Medicine, University of Alberta, Edmonton, AB, T6G 2G3.

ABSTRACT

Several species of bloom-forming cyanobacteria are known to produce highly toxic secondary metabolites. These toxins have been responsible for the poisoning and death of livestock and wildlife after ingestion of water or toxin-containing cells. Evidence of human poisoning is not as well documented but a substantial basis for concern exists. Cyanobacterial toxins are classified as either neuro- or hepato-(liver) toxins depending on their mode of action. This paper reviews the toxicology of the two main groups of toxins produced by freshwater cyanobacteria as well as providing data on the occurrence of one particular cyanobacterial hepatotoxin, microcystin-LR, in Alberta lakes. Our three year study has documented that microcystin-LR occurs frequently in eutrophic Alberta lakes. Concentrations of microcystin-LR in cyanobacterial blooms were sufficiently high on several occasions to warrant consideration of the potential health hazard. The concentration of microcystin-LR in the cyanobacterial bloom biomass was extremely variable both between lakes on the same day and from year to year within a given lake.

INTRODUCTION

Cyanobacteria (blue-green algae) commonly form surface blooms during the summer months in many productive lakes on the Canadian prairies. These blooms greatly reduce the useability of a water body by creating an aesthetic nuisance, tainting finished drinking water with musty odors and tastes and occasionally causing large summerkills of sportfish. Several species of cyanobacteria also produce contact irritants which result in skin rashes after swimming. These rashes are commonly lumped together into a much generalized and collective condition known as swimmer's itch. Cyanobacteria may present a health risk to livestock and wildlife. The potential health risk to humans is receiving considerable attention.

Several species of cyanobacteria that are common to Canadian prairie waterbodies are capable of producing highly toxic secondary metabolites known as toxins (Carmichael 1992). Cyanobacterial toxins can be much more potent than most pesticides and more potent than some notoriously toxic contaminants (e.g. 2,3,7,8-tetrachlorodibenzodioxin) (Table 1). More than a century has passed since the first recorded incident of mass mortality of domestic cattle associated with cyanobacterial poisoning (Francis 1878). Since then, many intermittent but repeated cases of such poisoning events have occurred worldwide involving livestock, wildlife and family pets. Death can occur quickly after ingestion of only a few mL of water and cells if the cyanobacterial bloom material contains sufficient toxin (Carmichael and Gorham 1977). Cyanobacterial toxins are mainly found within the cells but can be released to the water during bloom senescence or after treatment of the bloom with an algicide such as copper sulfate (Kenefick et al 1992). Poisonings and fatalities occur when animals consume water and/or toxin-producing cyanobacterial cells. No human fatalities have been documented in the literature, likely because of the reluctance of humans to drink water from areas containing cyanobacterial scums.

Table 1. Comparison of acute toxicities of various compounds.

Compound	Compound Class	Animal	Route*a	LD_{50}
Malathion	Insecticide	Rat	oral	2800 mg/kg
Toxaphene	Pesticide	Rat	oral	80 mg/kg
2,3,7,8-TCDD	Contaminant	Mice	oral	100-2500 µg/kg
2,3,7,8-TCDD	Contaminant	Mice	i.p.	100-600 µg/kg
Microcystin-LR	Cyanobact. Toxin	Mice	i.p.	50 µg/kg
Microcystin-LR	Cyanobact. Toxin	Mice	oral	3-4 mg/kg
Microcystin-LR	Cyanobact. Toxin	Rat	i.p.	70-120 µg/kg
Aphantoxin	Cyanobact. Toxin	Mice	i.p.	10 µg/kg

*a -i.p.=intraperitoneal injection
(Ware 1978; Carmichael et al. 1985; WHO 1989; Falconer et al. 1988; Miura et al. 1991)

However, serious human poisonings have occurred repeatedly after accidental ingestion of toxic cells and water. Long-term chronic exposure of humans to cyanobacterial toxins may also occur because conventional water treatment processes achieve only marginal removal of toxins from finished drinking water (Himberg et al. 1989). Advanced water treatment processes such as activated carbon filtration or ozonation are believed to be more effective at removing the toxins, but few cities and communities in Alberta possess such sophisticated treatment (Kotak 1991).

TYPES OF CYANOBACTERIAL TOXINS

Two types of toxic metabolites are produced by cyanobacteria: neurotoxins and hepato-(liver)-toxins. Ingestion of neurotoxins can lead to death in animals in as little as five minutes. Death is a result of paralysis of the muscles involved in breathing and respiratory arrest due to either neuromuscular blockade (Carmichael et al. 1975) or inhibition of cholinesterases (Cook et al. 1989), depending on the particular toxin. Two genera of cyanobacteria are responsible for the majority of neurotoxin-related poisonings: *Anabaena* and *Aphanizomenon*. *Anabaena flos-aquae* produces two neurotoxins termed anatoxin-a and anatoxin-a(s) with LD_{50}s of 200 and 50 µg/kg (ip.-intraperitoneal injections) respectively to mice (Carmichael 1988). *Aphanizomenon flos-aquae* also produces anatoxin-a and a more potent neurotoxin termed aphantoxin (LD_{50} =10 µg/kg, i.p. injection in mice). Aphantoxin is structurally similar to saxitoxin, the paralytic shellfish poison involved in red tide poisoning incidents. The production of aphantoxin by *Aphanizomenon* appears to be rare, having been confirmed only in New Hampshire, USA. Incidents of neurotoxic poisonings by *Anabaena* are commonly misdiagnosed because clinical signs of poisoning are similar to those produced by pesticide intoxication (Cook et al. 1989). More extensive reviews of cyanobacterial neurotoxins can be found in Carmichael et al. (1985) and Carmichael (1992).

Hepatotoxins are cited in the literature more often than neurotoxins in incidents of animal poisoning, both worldwide and in Canada, and as such will be discussed in more detail than the neurotoxins. Ingestion by, or injection into animals of hepatotoxin-producing cyanobacteria can lead to death of animals in less than 1 hour. Death is caused by extensive destruction of the liver (Kotak et al. in press). A single species of cyanobacteria may produce several hepatotoxins at one time. As many as twenty-four hepatotoxins, termed microcystins (named after *Microcystis aeruginosa*, the most common cyanobacterium capable of producing hepatotoxins) have been documented so far (Carmichael 1992). Table 2 lists the more commonly occurring microcystins. Microcystins consist of a cyclic peptide ring containing 7 amino acids. Our research group has been documenting the occurrence of one of the microcystins, microcystin-LR in productive lakes in Alberta. The -LR suffix denotes two amino acids (leucine and arginine) in the peptide which may vary to give different microcystin variants. Microcystin-LR has an LC_{50} of less than 50 µg/kg (i.p.) in mice (Kotak et al. in press).

Table 2. Properties of the most commonly occurring microcystins.

Toxin	Molecular wt.	LD_{50} -i.p. in mice (µg/kg)
Microcystin-LA	909	40
Microcystin-LR	994	50
Microcystin-YR	1044	68
Microcystin-YM	1035	56
Microcystin-RR	1037	600

(Elleman et al. 1978; Botes et al. 1982, 1985; Codd and Carmichael 1982; Carmichael 1988)

The toxicity of microcystin-LR to mammals has been studied more than other microcystins. Injection of laboratory animals with microcystin-LR results in clinical signs of poisoning including piloerection, lethargy, loss of coordination, pallor of the extremities and slow, labored breathing. Uptake of microcystin-LR from the bloodstream to the liver and resultant death can occur in as little as 40 minutes (Kotak et al. in press). Microcystin-LR causes rapid destruction of the endothelial lining of the liver and necrosis of hepatocytes, leading to a massive influx of blood into the liver from the rest of the body. Livers of mice injected with purified microcystin-LR can increase in weight by almost two-fold (Figure 1) due to the influx of blood. The ultimate cause of death is by hemorrhagic shock. Activities of liver-specific enzymes in the blood are commonly used as an index of cyanobacterial hepatotoxicosis. Structural damage to the liver by microcystin-LR causes a dramatic release of enzymes such as aspartate amino transferase (AST) and alanine amino transferase (ALT) from the liver to the bloodstream (Figures 2a+b). Such diagnostic tools are also helpful for characterizing cases of cyanobacterial poisonings in humans (Falconer et al. 1983).

Figure 1. Effect of injection of different doses of microcystin-LR on liver weight in mice. Six mice were used per dose. Control mice were injected with saline only. Vertical bars are standard errors of the treatment means.

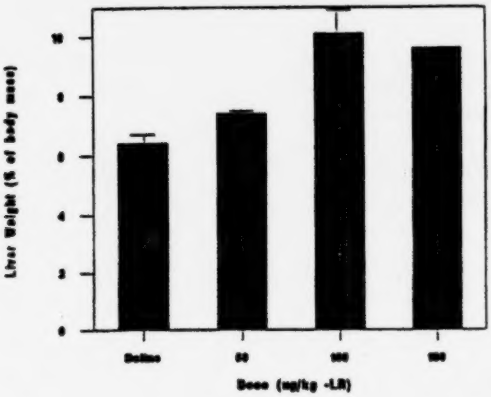
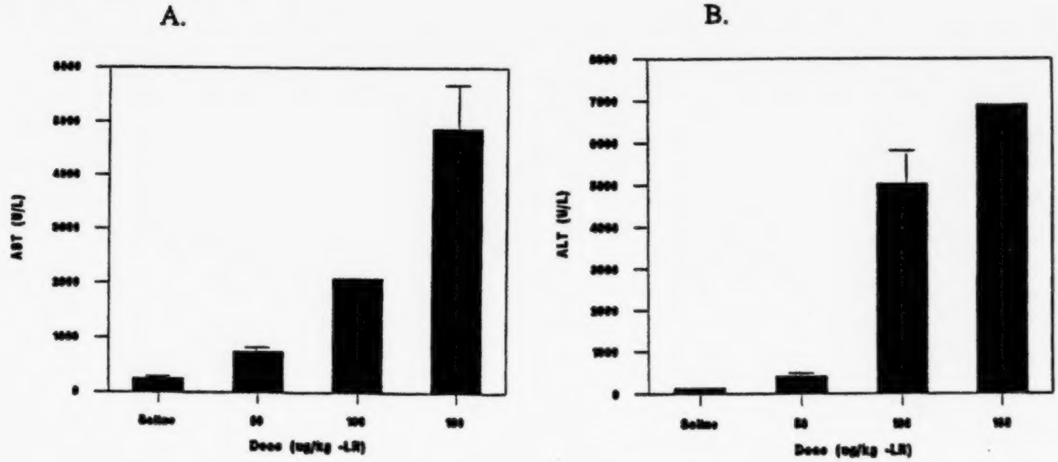


Figure 2. Effect of injection of different doses of microcystin-LR on a) blood serum activity of AST, and b) blood serum activity of ALT in mice. Six mice were used per dose. Control mice were injected with saline only. Vertical bars are standard errors of the treatment mean.



OCCURRENCE OF MICROCYSTIN-LR IN ALBERTA

Our research group has been documenting the occurrence of microcystin-LR in productive (i.e., eutrophic and hypereutrophic) lakes since 1990. We have monitored several lakes from May to October each year. Analysis of bloom samples by high performance liquid chromatography (HPLC) has indicated that microcystin-LR is produced in all study lakes either intermittently or on a continuous basis during the spring, summer and autumn months. Microcystin-LR is likely present in most of the productive lakes in Alberta. We have also detected microcystin-LR in farm dugouts utilized for drinking water, water which is not treated (beyond coarse filtering) prior to human consumption. In some cases, particularly in the lakes, concentrations of microcystin-LR were sufficiently high to warrant consideration of the potential health hazard.

Concentrations of microcystin-LR in the bloom biomass of our study lakes were extremely variable both temporally (throughout the spring, summer and autumn) in any one lake and spatially (between lakes) on the same day (Figure 3). The three lakes identified in Figure 3 are all drinking water supplies servicing a total population of more than 25,000. Coal Lake (servicing the city of Wetaskiwin, AB) had the highest concentrations of microcystin-LR in the blooms during 1990 while on average, Little Beaver Lake had the lowest. The timing of the period of maximum toxicity was also different between the three lakes. Coal Lake had highest microcystin-LR concentrations during late August when water temperatures were at their highest (24°C) while Little Beaver Lake had maximum microcystin-LR concentrations in late September when water temperature was only 10°C (Figure 3). Driedmeat Lake reached highest toxicity in mid September.

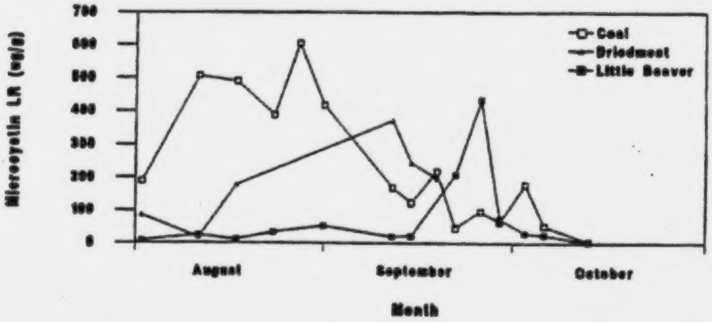


Figure 3. Seasonal variation in microcystin-LR concentration in the bloom biomass during 1990 in Coal, Driedmeat and Little Beaver Lakes, Alberta.

The literature commonly suggests that maximum toxicity (and therefore risk to animals) occurs during the warm summer months. Data from Little Beaver and Driedmeat Lakes suggest that water temperature may not be as important in toxin production as is commonly believed, although microcystin-LR production was strongly positively correlated to water temperature in Coal Lake during 1990 ($r=0.76$, $p<0.01$, $df=12$). Results from 1991 (data not shown) in Little Beaver Lake indicate highest toxicity (1307 µg microcystin-LR/g of bloom biomass) occurred on October 9 when water temperature was only 8°C. Microcystin-LR concentrations in blooms can also vary greatly between years within a lake. Figure 4 indicates substantial differences in microcystin-LR concentrations of the blooms of Coal Lake from 1990 to 1992. The concentration of microcystin-LR decreased over the three year study resulting in nondetectable levels throughout all of 1992. This decline in microcystin-LR production may be due to a species shift from *Microcystis* in 1990 to other cyanobacteria such as *Aphanizomenon*, *Lyngbya* and *Gomphosphaeria* in 1991 and 1992. This has not been tested yet as our phytoplankton samples are still being processed.

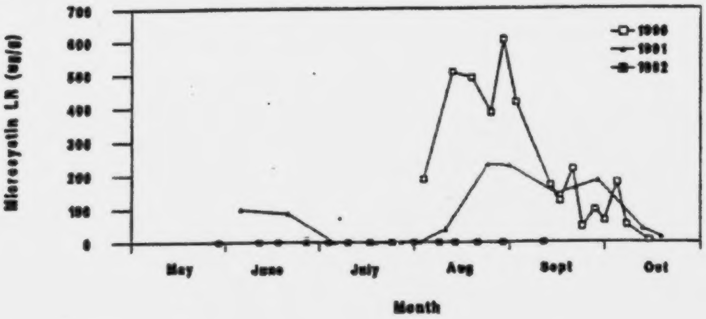


Figure 4. Year to year variation in the concentration of microcystin-LR in the cyanobacterial bloom biomass in Coal lake, Alberta.

Factors important in controlling toxin production in cyanobacterial blooms may include total phosphorus, total dissolved phosphorus, water temperature, Secchi depth, water pH and species composition of the bloom (Wicks and Thiel 1990; Kotak et al. in press). The literature also suggests that thicker (i.e., more intense) blooms of cyanobacteria produce higher toxin levels. Our data (not shown) do not indicate this. Higher chlorophyll a levels (an index of bloom biomass) are negatively correlated to microcystin-LR concentration. This is likely because the proportion of *Microcystis* in the blooms of our study lakes is often low and therefore higher bloom biomass (i.e., higher chlorophyll a levels) is usually attributed to other cyanobacterial species which are unable to produce microcystin-LR. Thus, the overall concentration of microcystin-LR per unit of bloom biomass decreases with increasing chlorophyll a. Therefore, thicker cyanobacterial scums on lakes do not necessarily represent a greater toxic risk.

CONCLUSIONS

Cyanobacterial toxins such as microcystin-LR are potent, naturally-occurring compounds produced by bloom-forming cyanobacteria in productive lakes. Microcystin-LR has been responsible for the poisoning and death of livestock and wildlife in Alberta and worldwide, and is under increasing scrutiny for its potential health risk to humans. Microcystin-LR is much more prevalent in productive Alberta lakes than previously thought. The high, acute toxicity of microcystin-LR to mammals and the repeated occurrence of poisoning incidents warrants the further investigation into the possible factors responsible for initiating and controlling microcystin-LR production in lakes. A health risk to users (swimmers and water skiers, drinking water users, cattle, wildlife) of lakes could exist. Guidance for levels of microcystin-LR that may be considered safe in lakes are urgently needed.

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A BIOENERGETIC APPROACH TO THE ANALYSIS OF CHRONIC STRESS ON FRESHWATER INVERTEBRATES. D.M.A. Monita, Dr. R.W. Davies, Department of Biological Sciences, The University of Calgary, 2500 University Drive N.W., Calgary, AB, T2N 1N4, (403) 220-5261.

A useful predictive bioassay must be a rapid, cost efficient, and, most importantly, accurate predictor of potential impacts of environmental stress in natural ecosystems. Currently, the acute lethality bioassay entrenched in Canadian and Provincial legislation does not provide the user with an accurate assessment of potential implications of environmental stress in aquatic ecosystems. A bioenergetic approach was utilized to investigate the effect on energy partitioning in organisms both during and after exposure to an environmental stress. The bioenergetic approach was evaluated for its suitability as a rapid, cost efficient and accurate predictive bioassay.

A bioenergetics model was developed to investigate energy acquisition and allocation patterns in a common and abundant freshwater macroinvertebrate, *Nephelopsis obscura*, in stressed and non-stressed environments. Cadmium was used as a stable and non-volatile heavy metal stress. All bioenergetic components including energy acquisition, absorption efficiency, energy assimilation, somatic and reproductive growth, respiration (maintenance and active), and energy storage were quantified and a comparison made between animals in stressed and non-stressed environments. Three concentrations of cadmium were applied to groups of animals at either their growth or reproductive stage in their life-cycle.

With increasing heavy metal stress, animals compensated for the effects of the stress by acquiring more energy and being more efficient at absorbing the energy from the ingested prey. The majority of the assimilated energy was allocated to maintenance respiration (which includes respiratory energy for repair). With increased concentrations of the stressor agent, the aerobic scope progressively decreased. Also, the animals had less energy available for activity and spent less time being active than that of the animals in the non-stressed environments.

During the period of stress, the animals exposed to the cadmium initially increased the amount of energy allocated to reproductive tissue, and more rapidly increased progression to further stages of reproductive development. However, after a period of time, the reproductive tissues degenerated, resulting in lower fecundity for the animals exposed to the stressed environments. Although all animals reached reproductive maturity at the same time, the animals in the stressed environments were significantly smaller. The animals in the non-stressed environments progressively increased the amount of energy allocated to reproductive materials throughout their life-cycle, were larger at time of maturity, and experienced higher fecundities.

Preliminary results showed that animals in non-stressed environments allocated energy to storage components, primarily lipids, and utilized the stored energy during reproduction. Animals in the stressed environments allocated less energy to storage during the initial part of the stress, but, towards the end of the stress period, they allocated no energy to storage and then began to utilize the stored energy.

Nephelopsis obscura showed higher sensitivity to stress when it was applied during the reproductive phase of its life-cycle, as compared to the growth phase. Notably, higher mortality rates were seen when the animals were exposed to stress during their reproductive phase. No animals exposed to the highest concentration of cadmium during their reproductive phase died within 96 hours (96 hour LC_{50} assays test for mortality during the first 96 hours of exposure only). There was, though, 100% mortality by the end of the stress period.

When a complete bioenergetic assessment is completed over the life-cycle for a native organism, a particular component of the equation may be a suitable predictor of allocation patterns. In these experiments, maintenance respiration was most closely correlated to the stressor agent, and could be used as an indicator of allocation patterns in the organism exposed to heavy metal stress. By utilizing only one component of the bioenergetic equation at one particular stage in the organism's life-cycle, the bioassay would provide a tremendous amount of information and prove to be both rapid and cost effective.

The bioenergetic assay would also allow a user to make accurate assessments of the potential implications of the discharge of an effluent into a receiving watercourse or waterbody based on energy acquisition and allocation patterns in a native organism. Decreased energy available for activity will result in decreased foraging and predator avoidance abilities. Longer termed demographic changes may also be predicted from the information on fecundity. The duration and timing of a discharge will also have implications to the long-term demography.

The bioenergetics approach to aquatic toxicity testing has the potential of being a rapid, cost effective, and comprehensive tool for assessing the effects of environmental stress in aquatic ecosystems.

BIOACCUMULATION OF HEAVY METALS IN GASTROPODS FROM LAKE SAINT-LOUIS (QUEBEC).

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The objective of our study is to evaluate the potential of the gastropods, organisms associated with the aquatic plants, in terms of bioindicators of the metal pollution in aquatic ecosystems. The main hypotheses of this study are: 1) the gastropods can be considered as bioindicators of the spatio-temporal variations of the metal contamination in soft water ecosystems; 2) the concentration of metals in the gastropods can be associated with the concentration of metals in the sediments, and 3) the trophic contamination by the seston, epiphyton or macrophytes influences the bioaccumulation of metals in the gastropods.

Samples of surface sediments and gastropods were taken in July and August 1991, at twelve sample stations in Lake Saint-Louis, a widening of the Saint-Laurent River. Each sample of sediments was submitted to a sequential extraction procedure in order to determine the distribution of trace metals within the different geochemical phases. The organisms were digested and analyzed for their content in Cu, Pb, Zn, Cd, Cr, Ni, Fe and Mn with flame atomic absorption spectrometer and graphite atomizer.

A statistical analysis is conducted in order to demonstrate the possible relation between the metal contamination of the sediments and the one observed in the gastropods.

A PHYSIOLOGICAL BIOINDICATOR AND THE PROBLEMS OF VALIDATION.

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The usefulness of bioindicators and their calibration and validation has become an important topic in environmental monitoring, worldwide. A number of physiological and biochemical parameters and measurements have been explored for use as indicators of environmental quality, and a number are currently being used for this purpose. In our investigations we have been measuring crustacean hemocyanin to determine its usefulness as a surrogate indicator of environmental quality. This approach is based on the hypothesis that hemocyanin turnover and synthesis is affected by extrinsic factors in the environment, and therefore, will reflect environmental quality.

Hemocyanin in blue crabs *Callinectes sapidus*, and Dungeness crabs, *Cancer magister*, as in all crustaceans is critical to their health, survival and normal physiological function, since it serves as the primary oxygen carrier in the hemolymph. Crustacean hemocyanin structure and function have been studied extensively. Hemocyanin is characterized as a large copper-containing protein composed of a minimum of six subunits each of 75 kDa molecular weight. The number of hexamers and their arrangement is species specific.

Extrinsic factors, such as salinity and dissolved oxygen concentration in the environment, influence the concentrations of hemocyanin in the hemolymph of certain marine crustaceans. Decreased salinity and low dissolved oxygen have been shown to cause an increase in hemocyanin in blue crabs. The information presented here suggests that hemocyanin concentrations may be also affected by chemical contaminants. Evidence that "water quality" as forcing function in the synthesis and turnover of hemocyanin in the blue crab and Dungeness crab has been documented, but the response patterns are not entirely predictable or well understood.

Hemolymph samples were collected from North Carolina blue crabs by the North Carolina Division of Marine Fisheries and from Straits of Georgia Dungeness crabs by the Institute of Ocean Sciences, Sidney, BC for the measurement of hemocyanin and the determination of its relation to water quality. Hemolymph was obtained from blue crabs by severing either the paddle appendage or a walking leg between the joints with a sharp pair of scissors and from Dungeness crabs using a hypodermic syringe and 18 guage needle. The samples were collected in plastic vials, placed on ice and allowed to clot. The samples were either sent directly to our laboratory on ice or frozen and shipped by air. The clotted material was broken up and then centrifuged at 20,000 x G for 30 minutes. The resulting serum was then decanted and kept at <4°C until measurements of hemocyanin were made. The hemocyanin measurements are made spectrophotometrically. The hemolymph serum samples are diluted with buffer and readings taken at 280 and 335 mn. These readings are compared to standards and the concentration of hemocyanin is calculated.

Blue crabs collected from the Pamlico River estuary in North Carolina during the summers of 1989, 1990, and 1991 have shown reduced hemolymph hemocyanin concentrations relative to adjacent reference areas. The overall differences in hemocyanin concentrations are significant, reference areas averaged about 45 mg/ml while the Pamlico sites averaged 15 - 28 mg/ml. The expected responses to lowered oxygen and reduced salinities, which is increased hemocyanin concentration, did not occur. At one site on the Pamlico River, where dissolved oxygen concentration and temperature data were available, hemocyanin concentration and lower oxygen were positively correlated and temperature negatively. It is our hypothesis that the combination of low dissolved oxygen and elevated temperatures caused the crabs to be less effective predators, and therefore, food limited which expressed itself as reductions in hemocyanin concentrations. Thus, the reduced hemocyanin was a secondary rather than a primary effect of low oxygen.

Blue crabs collected from Tampa Bay, Florida and the Houston Ship Channel, Texas showed a positive correlation between reduced hemocyanin concentrations and occurrence of organic chemical pollution. In the Tampa Bay crabs the measured organic contaminants were DDT and PCBs, and in the Ship Channel the main source of contamination was petroleum hydrocarbons. These data suggest, therefore, that hydrocarbon contamination may have directly affected hemocyanin and copper metabolism of the crabs that were examined.

Preliminary measurements were made of hemolymph hemocyanin concentrations in Dungeness crabs from a reference site and three pulp mill sites in the vicinity of the Strait of Georgia. The crabs collected from the sites near the outfalls of the three pulp mills, where reduced oxygen can be expected, had higher hemocyanin concentrations than the crabs collected from the reference site which is different from the Pamlico River blue crabs. If elevated temperature is a confounding factor with blue crabs, than in the waters of the Straits of Georgia where temperatures are relatively low, its effects would be negated. The lower temperatures would allow the crabs to respond to reduced dissolved oxygen concentrations by increasing their hemocyanin concentrations, which is what previous research would predict.

Our conclusion is that hemolymph hemocyanin concentrations can not be used as a bioindicator at this time. The reason is that many areas of uncertainty still exist concerning the metabolic control of hemocyanin turnover and synthesis in marine crustaceans. While this conclusion appears to be negative, further understanding of the extrinsic and intrinsic factors that regulate hemocyanin metabolism may allow the use of hemocyanin concentration as an indicator of environmental conditions.

ATMOSPHERIC TRANSPORT OF AGRICULTURAL HERBICIDES INTO SASKATCHEWAN SURFACE WATERS.

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The concentrations of seven herbicides were measured in the atmosphere and precipitation near Regina, Saskatchewan for two summers. The water and surface film of adjacent ponds were analyzed for the same herbicides. The interrelationships between the various environmental compartments will be discussed. The toxicological implications will be interpreted based on field and laboratory data.

EFFECTS OF AQUATIC TOXICITY ON THE BELUGAS OF THE ST. LAWRENCE ESTUARY.

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The population of beluga (*Delphinapterus leucas*) in the St. Lawrence estuary numbers about 500, reduced from the original population size of several thousand by harvest and control measures. Repeated surveys over a decade have not shown a measurable rate of increase, in spite of apparently abundant food stocks and absence of predation. Contaminant loads are known to be high, and pathological conditions, such as tumours and infections, are reported from stranded carcasses; a link with contaminants is often presumed. However, disease conditions are also found in cetaceans of other species stranded on other shores. Alternatively, contaminant loads are accused of affecting the reproductive capability of females. The observed proportion of grey (young) animals has been evaluated against a model of the dynamics of a beluga population, and found compatible with a stationary population, but not with population growth. The proportion of newborn calves, which varies from year to year, is large enough, in at least some years, to indicate a normal rate of reproduction. The validity of the evidence for implicating organic contaminants in the dynamics of this population, and of comparisons with Arctic populations, studied using different techniques, is reviewed.

Introduction

The beluga (*Delphinapterus leucas*) is an odontocete of the ice-adapted Arctic family Monodontidae. It is found all around the Arctic, associated in winter with marginal ice areas and in summer typically frequenting ice-free Arctic shorelines, where it is known for its habit of concentrating in estuaries, often seeking very shallow water. Its distribution also includes the sub-Arctic waters of Hudson and James bays; the extreme limit of the distribution of the species is a population that resides in the estuary of the St. Lawrence, Canada, at a latitude of 47-48 degrees.

This population has achieved a level of notoriety for a combination of factors: it is severely reduced from its original numbers; it is separated from its conspecifics; it carries a high load of contaminants; and it inhabits a restricted range in a river estuary the tributaries to which have been modified by dams, that carries heavy commercial and pleasure traffic, and that is subject to industrial pollution. The St. Lawrence beluga population has become a symbol of environmental activism.

The principal facts are that the population is contaminated and is greatly reduced from its original size. Simple post hoc, propter hoc argument is sometimes used to imply a relation (see, for example, Schafer et al. 1990, p. 414: "This decrease [from about 5000 to 350-500] appears to be related to an industrial pollution problem. . . If the rate at which the beluga population has decreased. . . over the last century continues, [its] disappearance. . . is conceivable.") Infections, tumours, and other pathological lesions are found in the stranded carcasses of naturally-dead belugas.

The object of this article is to show how the evaluation of the environmental effects of toxic pollutants, of which the presence in not in dispute, in such a system may be complicated by difficulty in determining the status of even such a highly-valued population, and further of unravelling the causes of its condition and separating the toxic effects of contaminants from other influences on it.

The original size of this population is unknown, although early travellers remarked on the large numbers of white whales inhabiting the St. Lawrence. With settlement of the St. Lawrence valley, the population was exploited, although it seems that early attempts to establish a whale fishery met with only limited success. However, from 1870 through 1945, the take estimated from records was 15645 (Reeves and Mitchell 1984, an average of 200/yr. Back-calculations of the population size, using harvest records, have led to estimates of the order of 5000+ for the population at some periods (Figure 1). The harvest records show large variations in the annual catch; for example, peaks in 1915 and in 1935 were apparently separated by a period in the middle 1920s when the harvest was a few tens per year. In the 1930s bounties were paid for beluga tails under the supposition that the beluga population was harmful to fish stocks. From 1932 to 1938, 2233 bounties were paid (but some, perhaps for tails of harbour porpoise *Phocoena phocoena*; see Laurin (1982) p. 23). Extermination was also attempted by bombing.

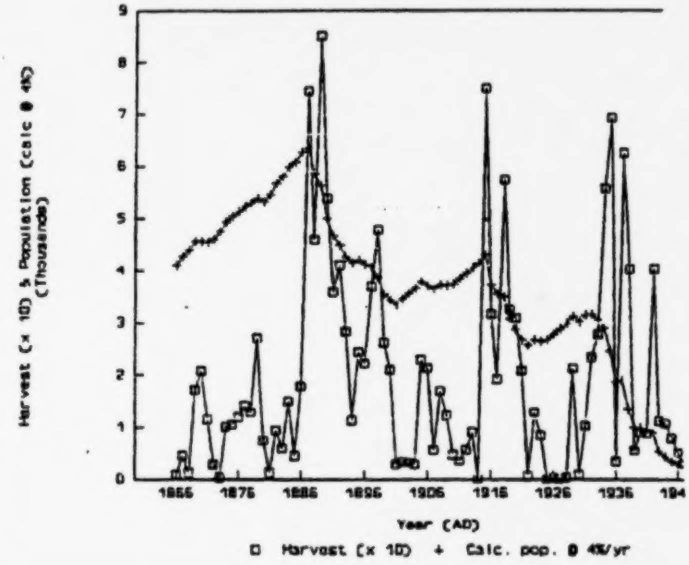

Figure 1. Harvest of St. Lawrence beluga (from Reeves and Mitchell 1984) and population estimates back-calculated from 400 in 1960 at 4% growth/yr.

In the late 1970s, concern was raised over the population when it was realised that its levels were low, that individuals carried high burdens of organochlorines and mercury, that its habitat was being disrupted by continued inputs of contaminants, riparian development, damming of inflowing rivers, and disturbance from a rapidly growing whale-watching industry as well as from other shipping, and that there was still no protection from hunting. Since then, the Beluga Protection Regulations, 1979 and 1980, gave the St. Lawrence beluga complete legal protection from hunting; the St. Lawrence Action Plan has worked on reducing contaminants and harassment.

Mechanisms suggested or implied, by which the environmental conditions may have reduced the population or its growth rate, have been that: (1) damming of rivers that flow into the St. Lawrence estuary has changed the water temperature and flow regime at their mouths, reducing the value to beluga of what was heavily-used habitat (Sergeant and Brodie 1975); (2) whale-watchers, whether commercial or private boaters, disrupt feeding, nursing, or other critical activities (Laurin 1982); (3) the immunosuppressive effects of toxic contaminants exposes the animals to higher risk of fatal infections (Béland et al. 1992); (4) contaminants induce fatal cancerous conditions (Martineau et al. 1985); (5) organochlorines disturb the hormonal functioning of the female reproductive system, or are fetotoxic (Martineau et al. 1987); (6) reduced-genetic diversity, due to the small size of the population, affects disease resistance by reducing the available diversity in the immune system; (7) reduced genetic diversity reduces reproductive success by duplicating genetic defects.

The symptoms of population malaise that have been cited or suggested include: (1) that the population recovered in the past from periods of heavy hunting that reduced it to low levels, but now is not recovering (Breton 1990, p. 29); (2) that the population continued to decline after the 1950s, for reasons other than hunting (Reeves and Mitchell 1984); (3) that the population was still declining in the 1980s, after full protection from hunting had been legislated (Pippard 1985; Massé et al. 1986); (4) that the population inhabits a range much reduced from its original range (Pippard 1985; Sergeant 1986; Sergeant and Hock 1988); (5) that the population has a lower gross rate of reproduction, and a lower proportion of juvenile animals, than Arctic populations (Sergeant 1986; Sergeant and Hock 1988); (6) that the population is unhealthy, indicated by monsters and deformities among live animals and by infections and tumours that are found in stranded carcasses more often than in the stranded carcasses of other cetacean species (Béland et al. 1992).

1. The population used to be able to recover from periods of heavy hunting that reduced it to low levels, but now does not (Breton 1990).

The documented data on the harvest levels from the population since 1868 show three major peaks, but even between them there is variation in the yearly take. "Assuming such variation or trends are not artifacts of documentation procedures but that they in fact reflect whale availability, these data imply . . repetitive. . depletion followed by recovery." (Reeves and Mitchell 1984). However, back-calculation models, even with the highest growth rates possible for beluga, show that the population could not track such variations (Figure 1). Either there was periodic immigration from Arctic populations, to an extent that the whole of the present population would be its offspring, or the variation in the annual take was due to fluctuations in reporting or interest, or to shifts in the distribution of the population that affected its susceptibility to harvest.

2. The population continued to decline after the 1950s, for reasons other than hunting, and was still declining in the 1980s, after full protection from hunting had been legislated. "The population is clearly declining, a trend which has been taking place over many years" (Pippard 1985, p. 442.); "Lately, this population has been declining" (Ray et al. 1991); "Whale hunting alone cannot account for the drastic decline of the beluga population" (Massé et al. 1986).

The status report prepared for COSEWIC (Pippard 1985) stresses the loss of critical habitat through such events as the damming of the Rivière Manicouagan and the Rivière des Outardes on the north shore of the St. Lawrence, rivers whose estuaries were formerly used by large numbers of beluga (Vladykov 1944), and the degradation of formerly favoured habitat such as Baie Ste Marguerite in the Saguenay fjord and Tadoussac Bay at its mouth by an increasing amount of small-boat traffic. The presence of toxic chemicals is mentioned, but less stress is laid on it.

Table 1. Population estimates for the beluga of the St. Lawrence

YEAR	AUTHOR	METHOD	EST.	INTERVAL	COMMENTS
Early 1960s	Montreuil	Aerial visual		1200-1500	(Pippard 1985); no information on design or coverage
1973	Sergeant & Hoek (1988)	Aerial photo.	443[c]	229-658 (95%)	Subjective sampling design; only covered high-density areas; low coverage
1977	Pippard (1985)	Aerial visual		300-350[u]	Complete count of high-density
1982	Sergeant & Hoek (1988)	Aerial visual	512[u]	360-715 (95%)	Random sub-sample from systematic transects
1984	Sergeant & Hoek (1988)	Aerial photo.	431[c]	187-773 (95%)	Systematic transect sample survey
1984	Lynas (1984)	Boat	495		Cited in Sergeant (1986)
1985	Sergeant & Hoek (1988)	Aerial photo.	530[c]	285-775 (95%)	Stratified systematic transect sample survey
1985	INESL (1987)	Boats	350		Complete count by 7 small boats
1987	Beland et al. (1987)	Aerial visual		436-487[u]	Systematic transects; intended as a distribution
1988	Kingsley and Hammill (1991)	Aerial photo.	491[c]	s.e. 69	Systematic transect sample survey
1990	ditto	ditto	607[c]	s.e. 308	ditto

[c] corrected for visibility

[u] uncorrected for visibility

Pippard (1985) cites a survey flown in the early 1960s as giving an estimate of 1200 to 1500 beluga between Quebec City and Les Escoumins, and other reports indicating similar totals in the late 1960s. Subjective opinions, among older residents, that there were more beluga in the St. Lawrence in the 1960s than there are now, may be heard in the area of the middle estuary. Aerial surveys in the 1970s and 1980s produced generally convergent results and estimates of the population size (Table 1). Those surveys with a satisfactory design, method, and effort seemed to agree on a range of estimates of 430-530; the agreement between surveys is closer than would be expected from some of the calculated confidence intervals. Depending on the reliance placed on anecdotal accounts of numbers in the 1960s, it seems likely that the population was greater in the 1960s than in the late 1970s and 1990s.

The documented series of statistics on the commercial catches of beluga in the St. Lawrence ends in 1975 (Laurin 1982, footnote to pg. 1), although Reeves and Mitchell (1984, Table 1) shows no data beyond 1960, and several years of "no information" in the 1950s. They indicate some exploitation by weir fisheries after 1960 (pg. 101), while an artisanal fishery, shooting from boats, was still being prosecuted on the north shore. Twenty to twenty-five may have been killed per year from 1961 on through the 1970s (Laurin 1982, pg. 24; Pippard and Malcolm 1978). If the permissible exploitation rate of monodontid populations is between 3%/yr (Kingsley 1989) and 5%/yr (Sergeant 1981), a population of 500 to 800 could have sustained these catch rates, but it must be emphasised that data on the catches is lacking, and they could have been larger. The hypothesis that residual hunting was controlling, or reducing, the population throughout the 1960s and 1970s, even in the absence of other effects, remains possible. The beluga protection regulations (P.C. 1980-355 SOR/80-376, p. 1944) legislated protection from hunting for the St. Lawrence beluga; whether all hunting would have ceased directly the law was promulgated it is difficult to say.

The recent data does not indicate a continued decline of the population; nor does it definitely indicate an increase. Monodontid populations may only be capable of mean rates of increase as low as 2 to 4%/yr (Béland et al 1988; Kingsley 1989; Doidge 1990), with unknown year-to-year variations in the realised rate. The sampling and measurement errors in available survey techniques for marine mammals are several times greater than that.

4. The population inhabits a range much reduced from its original range (Pippard 1985; Sergeant 1986; Sergeant and Hoek 1988): "the distribution was limited to a 50-km length of river centred on the reefs around the Saguenay River" (Sergeant and Brodie 1975).

Sergeant's surveys in the early 1980's supported his 1973 observation that the beluga range in the St. Lawrence was much reduced, and he concentrated his survey efforts on the area between Kamouraska and Les Escoumins. The reduced range may be a consequence, rather than a cause, of the reduced population. The year-round range of the population is now rather greater than shown in Pippard (1985, Figure 2). The upstream limit of regularly-used spring, summer and fall habitat lies near the Battures des Loups-Marins, and beluga are seen as far upstream as Ile aux Oies, or, infrequently, Quebec City. In winter beluga range at least as far downstream as Sept-Iles on the north shore and Cloridorme on the Gaspé side (Michaud 1991, Kingsley, unpublished data). But the substantial numbers of beluga that used the Manicouagan banks in the 1940s, and supported a fishery (Vladykov 1944), are still not seen there in the summer.

The population is not using the same areas in the same densities as it did fifty years ago. It is not clear whether the changes to the Manicouagan and Outardes river outflows have reduced or are controlling the population, whether they have altered the movements and behaviour (especially spawning), of prey fish stocks (which remain poorly understood), or whether alternatively the reduction of the population by other means, particularly hunting, has caused less favoured areas to be abandoned. The population shows no signs of overstocking in the range now available to it and being used by it.

5. The population has a lower gross rate of reproduction, and a lower proportion of juvenile animals, than Arctic populations (Sergeant 1986; Sergeant and Hoek 1988; Béland et al. 1992).

Two questions arise in this context: that organochlorine pollutants derange the hormonal control of the female reproductive system, and reduce pregnancy rates; and consistently with this, the reproductive rate and the proportion of young in this population are lower than in uncontaminated Arctic populations.

Marine mammals occupy high levels in what are usually long food chains, are long-lived, and have fat stores of which a large part is seldom mobilised. Unmobilised fat acts as a permanent reservoir for organochlorines. So they easily accumulate large organochlorine burdens. That the beluga of the St. Lawrence carry high burdens of organochlorine contaminants, and of some metals, is not in dispute (Massé et al. 1986; Martineau et al. 1987; Muir et al. 1990); however, stranded carcasses of naturally-dead animals may not be comparable with hunter-killed specimens (Wagemann et al. 1991). Possible prey species--fish in the maximum turbidity zone of the estuary have total PCB burdens of the order 0.1 ppm wet weight; zooplankton has about 0.01 ppm (Gagnon et al. 1990). Prey farther downstream in the middle estuary or the Gulf, may carry lower burdens; herring in the middle estuary is indicated as having 0.02 to 0.2 ppm PCBs wet weight (Khalil cited in Sergeant 1988). The degree of contamination of many of the organisms in the St. Lawrence estuary is low, or poorly known. Circumstantial evidence (the presence of Mirex in beluga, for which other sources are insufficient to explain loads) implicates the American eel, *Anguilla rostrata*, which migrates downstream at maturity from the more polluted areas of the upper St. Lawrence into the belugas' habitat. This species has a history of high organochlorine burdens (~6 ppm in 1986) and its migration could have carried downstream roughly twice as much PCBs as particulate matter in the water column (Cossa 1990). Contaminant levels in eels are now about 33% of their values in 1982 (Hodson et al. 1992).

Martineau et al. (1987) tabulated experimental results and circumstantial data indicating reproductive impairment in mammals related to organochlorine intake. Seals (Reijnders 1986) have furnished experimental evidence for marine mammals of reproductive dysfunction associated with diets high in OCs. A sample of female harbour seals fed contaminated fish had lower pregnancy rates than a control sample. PCB feeding rates for the treated group were of order 0.02 ppm body weight per day. Experimental results for mink-like seals, carnivores with delayed implantation indicated that trouble occurred at or soon after implantation. There has not been a controlled experimental study on belugas or other cetacean species of the effect of feeding contaminated diets. To have the same dosage rate (~0.02 ppm body weight/day) as Reijnders's treated group, if they fed only on the species tested by Gagnon in the maximum turbidity zone, beluga would have to eat about 20% of body weight per day; a more likely feeding rate (≈3% body weight/day) would give them similar dosage rates to Reijnders's control group, but would not explain the whole-body burdens observed in adult beluga.

The observed burdens of organochlorines are obviously not beneficial to the population, but in the absence of information on the diet and ingestion, it is not clear what effects they could be expected to have. Addison (1989) reviewing the connection between organochlorines and reproductive disorders in marine mammals, pointed out that cetacean populations in other areas have been reported as carrying similar burdens (without, however, presenting information on their status or dynamics. He concluded that the results on the St. Lawrence beluga were inconclusive in implicating organochlorines with reduced reproduction.

Absolute estimation of the proportion of young animals in a wild population is always subject to measurement errors. Young animals are never as visible as the adults, and often stay so close to their mothers that they are hard to pick out. The problem is compounded by sampling errors. The young themselves, and accompanying adults, behave differently from other adults, and frequent different areas of the range. In beluga, females with young appear to favour river estuaries in summer (Sergeant and Brodie 1975, p. 1047), and proportions of young may therefore be greater in such areas. In the St. Lawrence, it is well known that large adult males form a separate herd with a distinct range in deeper, colder water downstream off Les Escoumins, while females and young of all ages are found farther upstream.

The reproductive rates of beluga populations have been estimated by counting young animals from shore observation points, from boats, from visual aerial surveys, and from aerial photography. Calves of the year are identified by their small size, dark grey colour, and typical manner of surfacing. Other sub-adults are identified by their lighter, but still distinctly grey, colour (Béland et al. 1988). There is a cline in the visibility of grey coloration. It is more easily seen on dead beluga on shore than from boats, while in aerial photographs, animals of almost any age can hardly be described as grey. Even in visual aerial surveys, colour is hard to separate. Young must be picked out as being shorter. It is then difficult to separate calves from other young, as they grow only about 30 cm/yr. Reproductive rates have also been estimated by examining the pregnancy state of females taken in hunts and harvests. By reconstructing an intensively studied beluga population in NW Alaska, Burns and Seaman (1985) estimated calf production at 9-10%/yr, the non-calf grey animals (i.e. age classes I through 5) at 27.6%, and the total proportion of animals in the first six age classes at 35-37%; i.e. 63-65% mature.

The proportion of calves (age zero) in populations in the Canadian Arctic has been estimated at from 12 to 17% and the proportion of all grey animals, including calves, from 30 to 50% (Sergeant, 1986, Table IV; Table 2). These estimates were not verified against a model or reconstruction of a population, and seem high when compared with Burns and Seaman's (1985) estimates, or with the modelling results of Béland et al. (1988). Observations from the shore, and even aerial surveys near shore, might be expected to have higher proportions of females and young, if males favour areas further off-shore; this may be true even if the Churchill hunt selected a high proportion of males. Several of the results cited by Sergeant (1986) were made in the estuary of the Cunningham River on Somerset I; as noted below, they may have an excess of young.

Table 2. Estimates of proporations of calves in beluga populations (from Sergeant and Hoek 1988 and Burns and Seaman 1985).

SITE	YEAR	METHOD	CALF PROP'N
St. Lawrence	1983	visual aerial	0.083 Confusion between 0 and 1 yr. olds
St. Lawrence	1986	photo. aerial	0.055 Uncorrected for differential visibility of calves
St. Lawrence	1983	Ground obs'n site	0.051 Corrected for adult herd
Arctic (Hudson B., Cunningham Inlet)		Various ground counts, aerial survey	0.10 to 0.16 Possible bias due to inshore observations
Alaska		Population reconstruction	0.09

By contrast, Sergeant and Hoek (1988) explicitly corrected a counted proportion of calves made at Ile aux Lièvres in the St. Lawrence for the known existence of the downstream male herd. They also report a direct estimate of 5.5% calves from an aerial photographic (scale 1:1800) survey made in 1986. A proportion of calves will be missed in such a survey, because they are smaller and harder to see than the females. A correction was estimated from a photographic (scale 1:2400) survey flown with a high-precision camera with high frame overlap on colour transparency film in late July 1991 (Kingsley unpublished data). Of 18 occasions on which small calves (about half or less the length of the female) should have been visible on (an) adjacent frame(s), they were seen 11 times and not seen 7 times. Correcting Sergeant and Hoek's 1986 survey estimate (5.5% x 18/11) would give a 9% calf ratio--close to Burns and Seaman's figure. This correction is imprecisely estimated, the sample being small, but it is also minimum, as it assumes that visibility of calves is independent between neighbouring frames.

A further suggestion that the reproductive rate of St. Lawrence beluga is low was based on examination of reproductive tracts of stranded carcasses (Béland et al. 1992). While some of the carcasses showed evidence of recent reproductive activity, the proportion was low. However, the animals in question had all died of disease or old age, and many were beyond the age at which fertility starts to decline. It is not clear that such a sample can be validly compared with netted or shot samples.

Thus in comparing the Arctic calf production rates with the St. Lawrence, the following caveats ought to be applied: (1) population composition studies in near-shore Arctic areas may overestimate female/male ratios and young/female ratios for the whole population, owing to habitat segregation; (2) pregnancy rate estimates for the Arctic should be corrected for perinatal mortality to be comparable with calf counts; (3) calf counts by any method are underestimates; (4) production rates estimated from stranded naturally-dead animals would need to be validated by other means.

Kingsley and Hammill (1991) counted 23 (15.5%) small calves at heel on positive aerial photography flown in 1990 at a scale of 1:8000 over the whole range of the population. Reasonable assumptions on the visibility of calves in photography at such a scale, and on the age classes included in the classification of small calves at heel, would indicate that the calf production rate was not small in that year. A similar survey in 1988 yielded a much lower calf count (Kingsley and Hammill 1991).

6. The population is unhealthy, indicated by monsters and deformities among live animals and infections and tumours that are found in stranded carcasses of St. Lawrence beluga with greater frequency than in other species (Béland et al. 1992).

The stranded carcasses of dead belugas have been collected from the shores of the St. Lawrence, and examined since 1982. As well as basic biological data and tissue sampling for analysis for organochlorine and heavy metal contamination, carcasses in good condition have been necropsied in detail to discover pathological conditions and causes of death (Martineau et al. 1988, Beland et al. 1992). The findings have been that there is a high incidence of pathological conditions, particularly infections and tumours. It has been suggested that this is indicative of a low state of health in the live population in the St. Lawrence. The incidence of neoplasms reported in this population is higher than has been reported in other examinations of cetaceans. In 4 cetaceans and 23 pinnipeds for which there were non-human related pathological findings (out of 135 examined; there were large numbers of net entanglements and gunshot wounds), one cancer was reported (Deiter 1991). In 10 necropsied pilot whales (*Globicephala macrorhynchus*) that had died through mass stranding, one tumour was found; "moderate" or "severe" infections were found in half the group (Bossart et al. 1991). But the relationship of tumours to the presence of particular contaminants in the habitat or the individual is not clear. A reported bladder cancer was linked to emissions of PAHs in the area of upper Saguenay fjord (Martineau et al. 1985; Béland and Martineau 1988), but the etiological connection was questioned (Geraci et al. 1987; Geraci et al. 1988). The interpretation of reported pathological findings in marine mammals is the paucity of information on the age of the specimens.

An important factor in the pathology of St. Lawrence beluga is the fact that they have no significant cause of death: their food supplies are ample at most seasons, there is no reported predation on the population, they seem not to live strand, nor get caught in fishing gear, or hit by boats, or trapped in ice, and they are protected from being shot, harpooned, or trapped. The deaths of St. Lawrence beluga from disease are not necessarily a consequence of poor health in the population, they don't die of disease before anything else can get them, but a reflection of their immunity from other mortality factors.

The distribution of age at death for the stranded sample from the St. Lawrence is older than that of hunter-killed samples of beluga in northern Quebec (Figure 2); (The latter is an estimate of population age at death if hunting is a major source of mortality; probably true in this case). It is also older than the distribution of ages of dead stranded carcasses of other cetacean species in the St. Lawrence. Beluga are reported to show a decline in reproductive activity in older age classes, and the age distribution of dead stranded beluga is sufficiently old to indicate that even if the pathological conditions found were due to contaminants, the effect on the dynamics of the population is small.

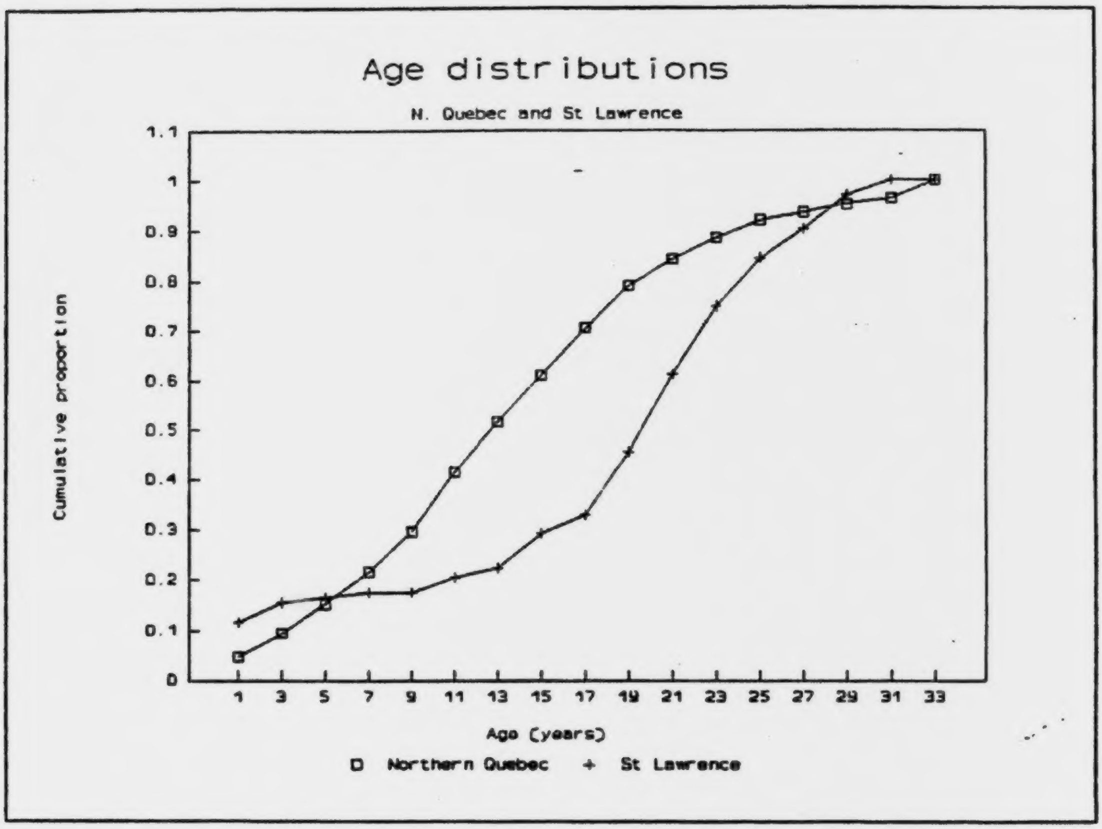

Figure 2. Distribution of ages from St. Lawrence stranded carcasses (Beland et al.) and from Nastapoka hunter-killed samples (Doidge 1990).

Cases of spinal curvature are known in recognised living members of the St. Lawrence population; such occurrences have not been reported in other beluga populations, either from live observations or hunter-killed samples. A carcass was reported with bilateral hermaphrodity (Béland et al. 1992).

Conclusion

The St. Lawrence beluga population rests a source of concern owing to its small size, the restricted habitat range, in a heavily travelled and industrialised estuary, that it uses, and its separation from its conspecifics. Its history presents a classic picture of over-exploitation, with successive cycles of heavy harvest meeting with less and less success until the population was finally fished to near-extinction.

The coincidence in time of a maximum of organochlorine pollution and a minimum size for this population led to an assumption that they were related. However, the evidence for continued decline of the population after exploitation was definitively stopped is slight. Growth of beluga populations is naturally slow, and would be expected to be hard to detect. Contaminants do not seem to have increased mortality, as the distribution of ages at death of recovered carcasses of St. Lawrence beluga shows long lives. The evidence for reduced reproductive rates is unconvincing, but there may have been a reduction in calving rate in the 1970s and 1980s. The investigation of calving rate remains a priority for the population.

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HISTOPATHOLOGICAL EFFECTS OF EXPERIMENTAL ACIDIFICATION ON LARGEMOUTH BASS, ROCK BASS, AND YELLOW PERCH FROM LITTLE ROCK LAKE, WISCONSIN.

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ABSTRACT

Little Rock Lake is a small softwater lake in northern Wisconsin, the two basins of which were separated by a plastic curtain in 1984. One basin was acidified to near target values of pH 5.6 in '85,'86; pH 5.1 in '87,'88; and pH 4.7 in '89,'90. The other basin served as a reference at pH 6.1. No pathology was seen in the gills of any species at pH 5.6. Statistically significant (relative to reference basin fish, t test, $p \leq 0.05$) gill changes occurred in all species at pH 5.1 and 4.7. These changes included: 1) alterations in chloride (ionoregulatory) cell numbers in rock bass and some perch at pH 5. 1, and in all species at pH 4.7; 2) decreases in mucous cell numbers in rock bass and some perch at pH 5.1, and in some perch at pH 4.7; 3) thickening of the respiratory epithelium (blood-water barrier) in largemouth bass at pH 4.7; and 4) moderate hyperplasia of the epithelium near the bases of respiratory lamellae in rock bass at pH 4.7. Pre-spawning ovaries of rock bass had significantly more immature, and significantly fewer late vitellogenic, follicles at pH 4.7. Scale Ca levels were low in largemouth bass juveniles during their last year of survival (1988, pH 5.1). With regard to gill pathology, rock bass were the most sensitive species, followed by largemouth bass and perch.

INTRODUCTION

Little Rock Lake (LRL) is a 18 hectare softwater seepage lake in northeastern Wisconsin, the two basins of which were separated by a vinyl curtain in 1984. One basin (treatment) was progressively acidified to near target values of pH 5.6 in '85, '86; pH 5.1 in '87, '88; and pH 4.7 in '89, '90. The other basin served as a reference at pH 6.1 (Eaton et al. 1992; Brezonik et al. 1993). The three most common species of fish in Little Rock Lake (LRL) are yellow perch, *Perca flavescens*, largemouth bass, *Micropterus salmoides*, and rock bass, *Ambloplites rupestris*. These species are considered highly (perch) to moderately (largemouth bass, rock bass) acid tolerant (Harvey, 1982; Rahel and Magnuson 1983).

For LRL rock bass and largemouth bass there were reductions in year-class strength at pH 5.6 and 5.1 due to reduced embryo-larval survival and/or juvenile recruitment (Eaton et al. 1992; Brezonik et al. 1993). Perch populations seemed to be unaffected at these pHs. At pH 4.7, there were year-class failures of rock bass and largemouth bass; young-of-year perch did not survive the second summer at pH 4.7 (Brezonik et al. 1993).

The primary goal of the present study was to determine if histopathological changes which may accompany acidification and contribute to declining fish health, occurred in samples of LRL fish (fish which were often obtained, not on the basis of preferred sampling times and ages, but secondary to other project goals). We were also interested in learning if there were histopathological changes which preceded, and therefore, might be used to predict, impending declines in fish populations, and if histopathology in LRL fish was similar to that found in limited, 30 day laboratory exposures (Leino et al. 1987a; McCormick et al. 1989a) involving juveniles of the particular species.

The histopathology studied relates primarily to the gills, the major target organ of acidification (From 1980; McDonald 1983). Ovaries of rock bass were also obtained for 1988-1990, when rock bass seemed to be experiencing reproductive problems. Finally, scales of young-of-year largemouth bass were obtained for 1988, the last year that juvenile largemouth bass survived, to determine if there were treatment-related differences in scale calcium levels. Scale Ca may decline in other situations, such as starvation or overwintering, where there is ionoregulatory stress (Ichikawa 1953; Leino and McCormick 1993).

Criteria for Gill Examination

Gills may exhibit a variety of histological changes when exposed to acid conditions. The criteria used in the present study were derived mainly from studies of fish exposed to acid in the laboratory, but also from whole ecosystem acidification studies (Chevalier et al. 1985; Leino et al. 1987b):

1. Chloride (ionoregulatory) cell numbers: Changes in chloride cell numbers may occur in gills of fish exposed to salt water, very soft water, or acidified water, presumably as a reaction to ionoregulatory stress (e.g., Leino and McCormick 1984; Chevalier et al. 1985; Laurent et al. 1985; Leino et al. 1987a, b; Tietge et al. 1988; Leino et al. 1990; Wendelaar Bonga et al. 1990; Leino et al. 1992; Leino and McCormick 1993).

2. Chloride cells with apical pits: Increases in numbers of chloride cells with a unique alteration in morphology, in which the exposed surface of the cells invaginates to form epical pits, were observed in fathead minnows and pearl dace exposed to acidified water (Leino and McCormick 1984; Leino et al. 1987b; Leino et al. 1990). This is the most specific histological response associated with acid exposure found to date: other histological changes which may accompany acidification, such as gill hyperplasia, also occur with a variety of other gill irritants (Mallett et al. 1985).

3. Vacuolated chloride cells: Numbers of chloride cells with cytoplasmic vacuolation frequently increase with acid exposure and may represent damaged or dying chloride cells (Chevalier et al. 1985; Leino et al. 1987a).

4. Mucous, rodlet, and granular cell densities: Densities of other gill cell types sometimes change during acidification. In some cases the other cell types seem to be crowded out by chloride or pavement cell hyperplasia (Leino et al. 1987b). In some species mucous cells proliferate due to acid exposure (Daye and Garside 1976; Jagoe and Haines 1990).

5. Hyperplasia of the interlamellar epithelium: Interlamellar hyperplasia is sometimes associated with chronic acid exposure (Chevalier et al. 1985; Leino et al. 1987a,b; Leino et al. 1990; Brown et al. 1990). The hyperplastic epithelium covers the basal parts of the respiratory lamellae resulting in a decrease in respiratory capacity. Occasionally hyperplasia can become so severe that it obliterates the respiratory lamellae and bridges the gap between adjacent gill filaments, causing them to fuse (Leino et al. 1987b).

6. Fusion of respiratory lamellae: Tips of respiratory lamellae or entire respiratory leaflets may fuse as a result of acid exposure (Jagoe and Haines 1983).

7. Thickness of the blood-water (respiratory) barrier: An increase in respiratory barrier thickness may accompany acidification (Leino et al. 1987a,b; Leino et al. 1990) and is another factor which results in a decrease in respiratory capacity (Hughes and Perry 1976).

8. Presence of red blood cells in the respiratory capillaries: Reduced numbers of red blood cells in respiratory capillaries of acid-exposed fish have been reported (Leino et al. 1990). It is not known why this occurs. Possibly the increase in blood viscosity observed in some acid exposed fish results in a reduction of systemic blood flow (Wood and McDonald 1987), or there is damage to the gill microvasculature, or a reduction of blood flow to the respiratory lamellae is induced through some physiological mechanism to reduce ion losses.

9. Edema: Gill edema, sometimes accompanied by lifting or separation of the respiratory lamellar epithelium, may occur with acute acid stress (Jagoe and Haines 1983; Daye and Garside 1976; Leino et al. 1987a; Freda et al. 1991).

10. Mucification or exudation over respiratory surfaces: Increased mucus secretion, and leakage of plasma, presumably from damaged respiratory epithelia, may accompany acute acid stress (Jagoe and Haines 1983; Daye and Garside 1976; Leino et al. 1990).

A combination of various types of gill pathology such as those mentioned above probably reflects or contributes to the major cause of acid associated fish mortalities: damage to the gill ionoregulatory mechanisms resulting in ionoregulatory failure. The fish loses electrolytes and dies.

MATERIALS AND METHODS

Fish and Blood Osmolalities

Adults and juveniles, when available, of LRL largemouth bass, rock bass, and yellow perch were caught quickly by hook and line: this was found to minimize stress for blood osmolality determinations and to allow reliable osmolality readings. The caudal peduncles were severed and blood drawn with an Eppendorf pipette immediately after measurements of weights and lengths. The blood osmolality was usually obtained, using a Wescor osmometer, within a minute after the fish were caught. Ten fish per basin were typically used for osmolality determinations and five or six fish per basin for microscopic studies.

Tissue Preparation and Histopathology

Tissues were fixed in cold 2.5% glutaraldehyde - 2% formaldehyde in 0.08M phosphate buffer for at least 48 hours. They were then rinsed in phosphate buffer, dehydrated in graded ethanol solutions, and embedded in Dupont JB-4 methacrylate (Leino and McCormick 1984). Sections were stained with eosin followed by hematoxylin.

Cell counts

Gills were oriented with their medial hemibranches placed downward in embedding molds. Cell counts were made from 2 µm thick longitudinal sections of comparable areas on each medial hemibranch, i.e., from a 1000-1200 µm long portion of the filament lying between the efferent artery and the gill ray (diagram in Leino and McCormick 1984). This region includes the central part of the respiratory lamellae. All chloride and mucous cells in the filamental and respiratory epithelia were counted on both sides of the filament. Vacuolated chloride cells and those with epical pits were also counted. In some instances, other gill cell-types (rodlet and granular) were counted. Slides for all procedures were coded so that the evaluator had no knowledge of the basin from which the fish came. Cell numbers were normalized for filament length for statistical analysis with the t test at the $p \leq 0.05$ significance level.

Other gill histopathology

Using 3-4 sections of gill per fish, other gill pathology was estimated with a least to most scale using the coded slides (Leino et al. 1990): 1) thickness of the gas exchange surfaces (bloodwater barrier); 2) amount of hyperplasia of the interlamellar epithelium; 3) abundance of interlamellar mucus; 4) edema, necrosis, or sloughing of the epithelium; 5) interlamellar plasma and red blood cell exudate; 6) reduction in numbers of red blood cells in capillaries of respiratory lamellae; and 7) fusion of primary or respiratory lamellae.

Laboratory exposures

In some instances histological comparisons were made to gills of juvenile perch, largemouth bass, and rock bass exposed to acidified soft water in the laboratory (Leino et al. 1987a; McCormick et al. 1989; unpublished data).

Rock bass ovaries

Pre-spawning ovaries were obtained from LRL rock bass during 6-15 June 1988-1990, and prepared for histological examination in a similar manner to the gills. The ovaries were microscopically staged according to Leino et al. (1990) using coded slides.

Largemouth bass scale calcium

Examination of scales for signs of calcium resorption may be a simple way to assess calcium loss in stressed fish (Ichikawa 1953; Yamada 1956). Scales of young-of-year largemouth bass were examined for Ca content using von Kossa's stain for calcium salts (Lillie 1965). Differences in the percent of Ca stained area for 8 scales from the mid-lateral surface of six fish per basin were determined by computerized image analyses using coded slides.

RESULTS

Gills

Largemouth bass

Gills of juvenile LRL largemouth bass were examined at pH 5.6 and pH 5.1; only adult gills were obtained at pH 4.7 since there were no juvenile year classes. No gill pathology was discerned at pH 5.6 and little at pH 5.1 (Tables 1, 4). At pH 4.7, there were increases in chloride cell numbers and in numbers of chloride cells with apical pits as well as increases in thickness of the respiratory epithelia (cf Figures 1-2). These changes resembled those found in 30 day laboratory exposures (Table 1).

Rock bass

Gills of adult rock bass exhibited no pathology at pH 5.6 (juvenile rock bass are secretive and were impossible to obtain without disrupting other LRL experiments). At pH 5.1, chloride cell numbers were depressed (Tables 2, 4). At pH 4.7, numbers of chloride cells were depressed and there were higher numbers of these cells with apical pits and with cytoplasmic vacuolation compared to the reference basin gills. Also at this pH epithelial hyperplasia at the bases of the respiratory lamellae gave these lamellae a triangular profile in histological sections (cf Figures 3-4). Mean blood osmolality was significantly lower in the treatment basin at pH 4.7 in 1990 (Table 2).

Juvenile rock bass were used for the 30 day laboratory exposures. These fish exhibited some of the same gill changes at similar pHs to those of LRL (Table 2) but, with acid exposure, there was a consistent increase rather than a decrease in numbers of chloride cells.

Yellow perch

There was little acid-related gill damage in LRL perch (Tables 3, 4), even at the lowest treatment pH (4.7). Chloride cells of juveniles tended to increase in numbers in response to LRL pH reductions (cf Figures 5-6), while numbers in adults tended to be similar in the treatment and reference basins. Sometimes a small percentage of chloride cells exhibited epical pits. In the laboratory study, it was not until pH 4.1 that perch gills exhibited major pathological changes (Leino et al. 1987a).

Rock Bass Ovaries

In 1988, there were no significant differences in ovarian maturities between basins, although there was one case of extreme follicular atresia in the treatment basin (pH 5.1). In 1989+90, ovaries from the treatment basin (pH 4.7) had significantly more immature and significantly fewer late vitellogenic follicles (Table 5).

Largemouth Bass Scale Calcium

The areas of the scales which exhibited Ca staining in young-of-year largemouth bass sampled in August 1988, were not significantly different: 55% for the pH 5.1 treatment basin and 64% for the pH 6.1 reference basin. But these values were far lower than laboratory values at similar temperatures and pHs (Figure 7). Instead the scale calcium levels in the LRL largemouth bass resembled those from largemouth bass stressed by exposure to overwintering temperatures for prolonged periods in the laboratory (Figure 7; Leino and McCormick 1993).

DISCUSSION

With regard to the effects of acidification on gill pathology in LRL fish, using as the primary criteria: 1) a depression in chloride cell numbers 2) gill hyperplasia, and 3) a thickened respiratory epithelium, rock bass seemed to be the most vulnerable, followed by largemouth bass. Perch gills were the most resistant to changes associated with reductions in pH. These results are consistent with the observed acid sensitivities of these species in LRL (Eaton et al. 1992) and their presence or absence in lakes of different pHs (Rahel and Magnuson 1983). The present study also affirms that, with regard to gill pathology, the LRL species examined are moderately (rock bass, largemouth bass) to highly (perch) acid tolerant; the gills exhibited fewer and milder pathology at a given pH than did highly acid sensitive pearl dace and fathead minnows from experimentally acidified Canadian lakes (Leino et al. 1987b).

While gill pathology, i.e., pathology of the major target organ of acidification, seemed to be a good indicator of relative acid sensitivity of perch, largemouth bass and rock bass, it could not have been used to predict the year class failures of largemouth bass and rock bass which occurred in LRL, probably because of the limited sampling dates available to the present study. For example, little gill pathology was observed in juvenile largemouth bass at pH 5.6 and 5.1, yet there was decreased juvenile survival at these pHs (Eaton et al. 1992). According to our hypothesis, substantial gill pathology should have accompanied this reduction in survival. However, these mortalities occurred during the winter months when no histological samples were available. This was one factor which prompted a laboratory "overwintering" experiment (McCormick and Jensen 1992; Leino and McCormick 1993) which showed that prolonged exposure of juvenile largemouth bass to cold acidified water resulted in considerable gill pathology, a reduction in blood osmolality, and increased mortalities, particularly with moderately elevated Al levels similar to those in LRL.

In the present study, the observed gill changes were consistent with those seen in other studies (see "Criteria for Gill Examination" in introduction): changes typical of chronic exposure to acidification tended to occur while those typical of acute exposure tended to be absent. For example, epical pits appeared in some of the chloride cells of acid-exposed perch, largemouth bass, and rock bass but acute lifting of the respiratory epithelium and increased secretion of mucus were not observed. However, slightly greater gill pathology, and substantially higher levels of chloride cells occurred in LRL fish, especially rock bass and largemouth bass, than in our laboratory studies. This could be associated with the greater duration of exposure to acidic conditions (including the reference basin at pH 6.1) and to higher levels of potentially toxic cations (Al, Cd, Mn, Fe, Zn, Pb; see Brezonik et al. 1993) in LRL.

While gills of LRL and laboratory exposed fish exhibited many similarities in their responses to acidification, there were also differences. Most notable was the decrease in chloride cell numbers in acid exposed LRL rock bass adults, compared with an increase in numbers of these cells in laboratory exposed juveniles. The fact that chloride cell numbers may increase, decrease, or stay the same upon exposure of fish to acid has been noted previously (Leino et al. 1987a; Wendelaar Bonga et al. 1990). In the present example, some difference in the lake and laboratory treatments, e.g., duration of exposure to low pH, could have resulted in a different gill reaction. Alternatively, juvenile rock bass may react differently than adults to acidification. In our experience, moderate increases in chloride cell numbers as occurred in laboratory rock bass at pH 5.0 often indicate a successful adaptation to lowered pH: electrolyte balance is successfully stabilized, albeit sometimes at lower blood osmolalities (Leino et al. 1990). Declines in chloride cell numbers, on the other hand, may be related to an acid-induced abnormal balance in the life cycle of these cells, i.e., lower production or higher death rate. This may be physiologically significant: lowered chloride cell density would be expected to result in decreased ion uptake (Leino et al. 1987a; Wendelaar Bonga et al. 1990) as appeared to occur in LRL rock bass at pH 4.7.

Rapid, dramatic increases in chloride cell numbers subsequent to acidification in very soft water[1],as occurred in the laboratory rock bass exposed to pH 4.0, seem to be an acute reaction to rapid loss of ions. In the species we have examined, this type of chloride cell hyperplasia is a good predictor of high mortalities, due to loss of electrolytes, within several days (e.g., Leino et al. 1992).

The regressive changes observed in ovaries of pre-spawning rock bass at pH 4.7 suggests that these rock bass were beginning to experience problems with oogenesis. This observation is consistent with the abnormal spawning observed in LRL rock bass at pH 4.7: nests never lasted for more than a few days and no larvae were seen (Eaton et al. 1992).

Little research has been done on the significance and dynamics of Ca in scales. However, Ca is thought to be drawn, when needed, from reserves in scales (Ouchi et al. 1972; Mugiya and Watabe 1977), so examination of scales for Ca content may be a simple way of determining if a fish is undergoing Ca-related, and even ionoregulatory, stress (Ichikawa 1953; Yamada 1956; Leino and McCormick 1993). The relatively low levels of scale Ca found in LRL largemouth bass juveniles in 1988 in both reference (pH 6.1) and treatment (pH 5.1) basins, compared with laboratory exposures suggests that these juveniles may have had suboptimal Ca reserves. Low reserves of Ca may be a factor in the decreased fitness of juveniles entering the first winter at low pH, where additional stresses may further affect Ca uptake and utilization and contribute to the electrolyte problems associated with overwintering mortalities (McCormick and Jensen 1992; Leino and McCormick 1993).

We thank Kathleen Jensen, Deborah Pomroy-Petry, Tim Simonson, and Bill Swenson for their assistance. This study was supported by the USA Environmental Protection Agency (CR 814582-01-4).

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[1] In more ion-rich acidified water, numbers of chloride cells may increase to high levels and the fish maintains a high blood osmolality presumably because there are sufficient ions available for efficient ion uptake (Leino and McCormick 1984; Leino et al. 1990; Wendelaar Bonga et al. 1990). Survival under these conditions is much better than in acidified very soft water.

Table 1. Summary of gill pathology and mean blood osmolality in mosmol/kg (reference/treatment) in largemouth bass from the treatment basin of LRL based on comparisons with the corresponding reference (pH 6.1) basin fish, and contrasted with laboratory results.

LRL	LABORATORY EXPOSURES (9/84)
	pH 7.0, juveniles: no pathology 319*
pH 5.6, 9/86, juveniles: no pathology 297|301	
pH 5.1, 8/87, juveniles: no pathology 296|298	pH 5.0, juveniles: increase in number of chloride cells with apical pits thickened respiratory barrier 312*
pH 5.1, 9/88, juveniles: slightly thickened respiratory barrier 293|290	
pH 4.7, 6/90, adults: increase in numbers of chloride cells in primary lamellar epithelium great increase in number of chloride cells with apical pits thickened respiratory barrier	pH 4.5, juveniles: increase in number of chloride cells in primary lamellar epithelium increase in number of chloride cells with apical pits thickened respiratory barrier 311*

* McCormick et al. 1989

Table 2. Summary of gill pathology and mean blood osmolality in mosmol/kg (reference/treatment) in rock bass from the treatment basin of LRL based on comparisons with the corresponding reference (pH 6.1) basin fish, and contrasted with laboratory results.

LRL	LABORATORY EXPOSURES (4/85)
	pH 7.0, juveniles: no pathology 294*
pH 5.6, 7/85, adults: no pathology 289|289 (6/86 sample)	
pH 5.1, 6/88, adults: decrease in number of chloride cells in gill epithelium (primary and respiratory lamellae) 294|296 (5/87 sample)	pH 5.0, juveniles: increase in number of chloride cells in gill epithelium increase in number of chloride cells with apical pits possible thickened respiratory barrier 284*
pH 4.7, 9/89, adults: decrease in number of chloride cells and mucous cells in gill epithelium 278|271	pH 4.5, juveniles: increase in number of chloride cells in gill epithelium transient increase in number of chloride cells with apical pits possible thickened respiratory barrier "triangular" lamellar hyperplasia 269*
pH 4.7, 6/90, adults: decrease in number of chloride cells primary lamellar epithelium increase in number of chloride cells with apical pits increase in number of vacuolated chloride cells "triangular" lamellar hyperplasia (see text) 289|263**	
	pH 4.0, juveniles: rapid increase in number of chloride cells in gill epithelium increase in number of chloride cells with apical pits possible thickened respiratory barrier "triangular" lamellar hyperplasia 174* (15 d at pH 4.0; all dead at 30 d)

** Significantly different from reference basin, t test, $p<0.05$

* McCormick et al, 1989

Table 3. Summary of gill pathology and mean blood osmolality in mosmol/kg (reference/treatment) in yellow perch from the treatment basin of LRL based on comparisons with the corresponding reference (pH 6.1) basin fish, and contrasted with laboratory results.

LRL	LABORATORY EXPOSURES (6/84)
	pH 7.0, juveniles: no pathology 322*
pH 5.7, 7/85, adults: no pathology 297|293	
pH 5.1, 8/87 adults: no pathology 297|297	pH 5.0, juveniles: no pathology 306*
pH 5.1, 4/88, adults: increase in number of chloride cells in gill epithelium	
pH 5.1, 9/88, adults: moderate hyperplasia of primary lamellar epithelium moderately thickened respiratory barrier moderate gill edema 289|289	
pH 5.1, 6/88, juveniles: no pathology; fewer mucous, rodlet, and granular cells	
pH 4.7, 9/89, adults: fewer mucous, rodlet, and granular cells 281|286	
pH 4.7, 9/89, juveniles: increase in number of chloride cells in gill epithelium increase in number of chloride cells with apical pits thickened respiratory barrier due to hypertrophic chloride cells in respiratory epithelium fewer mucous, rodlet, and granular cells 292|293	
pH 4.7, 6/90, adults: increase in number of chloride cells with apical pits	
pH 4.7, 10/90, juveniles: increase in number of chloride cells in gill epithelium increase in number of chloride cells with apical pits 296|292	pH 4.1, juveniles: decrease in number of chloride cells in primary lamellar epithelium increase in number of chloride cells with apical pits hyperplasia of primary lamellar epithelium thickened respiratory barrier 252*

* Leino et al. 1987a

Table 4. Some key gill measurements from LRL largemouth bass, rock bass, and perch.

Fish, maturity, date	Basin, N fish	Cloride cells per cm ± SEM	% CC's with apical pits	Blood-water barrier width 0-+++, (μm)	Hyperplasia of primary epithelium[1]
LMB, juv, 9/86	REF 6 TRT 6	671±96 864±98	1.4 1.8	0 0	0 0
LMB, juv, 8/87	REF 10 TRT 9	738±89 506±76	1.9 1.3	+ (3.7) + (3.0)**	++(27.4) + (19.1)**
LMB, juv, 9/88	REF 5 TRT 5	223±20 213±76	0 0	0 (1.3) + (1.3)	0 (24.3) 0 (26.5)
LMB, adult, 6/90	REF 4 TRT 3	704±189 1179±187*	1.0 5.4**	0 (3.9) + (8.2)**	0 (27.9) 0 (30.1)
RB, adult, 7/85	REF 6 TRT 6	553±103 327±67	0 0	0 0	0 0
RB, adult, 6/88	REF 6 TRT 6	519±87 277±67*	0 0	0 (3.2) 0 (2.7)	0 (23.5) 0 (16.9)
RB, adult, 9/89	REF 6 TRT 6	643±113 323±40*	0 0	+ 0	+ 0
RB, adult, 6/90	REF 6 TRT 4	1120±88 935±81*	0.3 1.0**	0 (2.5) + (3.0)	0 (25.9) 0 (25.2)
PER, adult, 7/85	REF 5 TRT 5	944±119 1009±80	1.2 0.9	0 0	0 0
PER, adult, 8/87	REF 6 TRT 5	841±197 930±233	1.6 1.9	+ (3.4) 0 (2.7)	+ (29.3) 0 (27.1)
PER, juv, 6/88	REF 4 TRT 6	1446±244 1605±199	0 0.4	0 0	0 0
PER, adult, 4/88	REF 5 TRT 5	609±66 1074±203*	0 0	0 0	0 0
PER, adult, 9/88	REF 5 TRT 5	862±162 753±128	1.4 1.1	0 0	0 +
PER, juv, 9/89	REF 8 TRT 10	1351±165 1974±96*	0.6 1.1**	+ ++	+ 0
PER, adult, 9/89	REF 5 TRT 5	426±83 464±186	0.9 1.2	0 0	+ 0
PER, adult, 6/90	REF 5 TRT 5	1726±201 1614±280	0.1 0.4**	+ +	0 +
PER, juv, 10/90	REF 6 TRT 6	873±73 1334±112*	1.0 3.4**	0 0	0 0

[1] ( ) width of primary lamellar epithelium in μm

* significantly different from REF basin, t test, $p \leq 0.05$; see tables 1-3 for population of chloride cells involved, i.e., total chloride cells, primary lamellar chloride cells

** numbers of cells or measured thickness significantly different from REF basin, t test, $p \leq 0.05$

Table 5. Mean percent of various follicular stages in ovaries of LRL rock bass in 1988-1990.

STAGE	REF BASIN	TRT BASIN
1988	(pH 6.1)	(pH 5.1)
Immature follicles	68.0	64.7
Early vitellogenic follicles	14.9	18.3
Late vitellogenic follicles	17.2	17.0
Total vitellogenic follicles	32.1	35.3
[1]Atretic: vitellogenic follicles	1.3	18.2[2]
1989 + 1990		(pH 4.7)
Immature follicles	60.0	73.5*
Early vitellogenic follicles	22.7	16.9
Late vitellogenic follicles	17.3	9.6*
Total vitellogenic follicles	40.0	26.5
[1]Atretic: vitellogenic follicles	1.8	3.5

[1] Percent atretic follicles=number of atretic follicles/total vitellogenic follicles*100

[2] High value results from one fish with extreme follicular atresia

* Significantly different from REF basin, t test, $p<0.05$

NOTE: N=5, except 1988 TRT sample where N=6

Figure 1. Section of gill from juvenile largemouth bass (laboratory experiment), pH 7.8, showing healthy gill structure typically found at circumneutral pHs. 314x

1

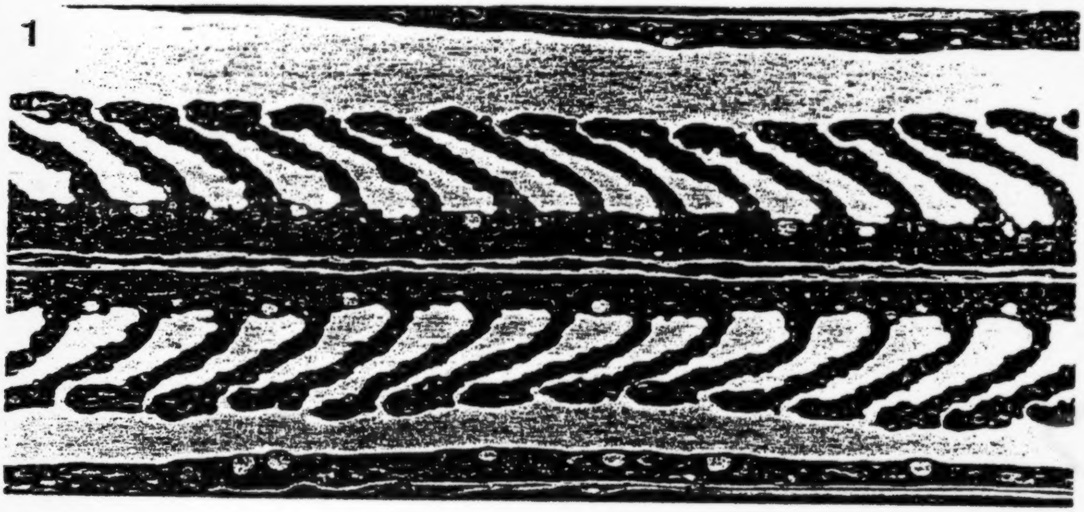


Figure 2. Section of juvenile largemouth bass gill from the treatment basin, June, 1990, pH 4.7, showing thickened epithelium of the respiratory lamellae (R) primarily due to chloride cell (C) hyperplasia. Note chloride cell with apical pit (arrowhead). 475x

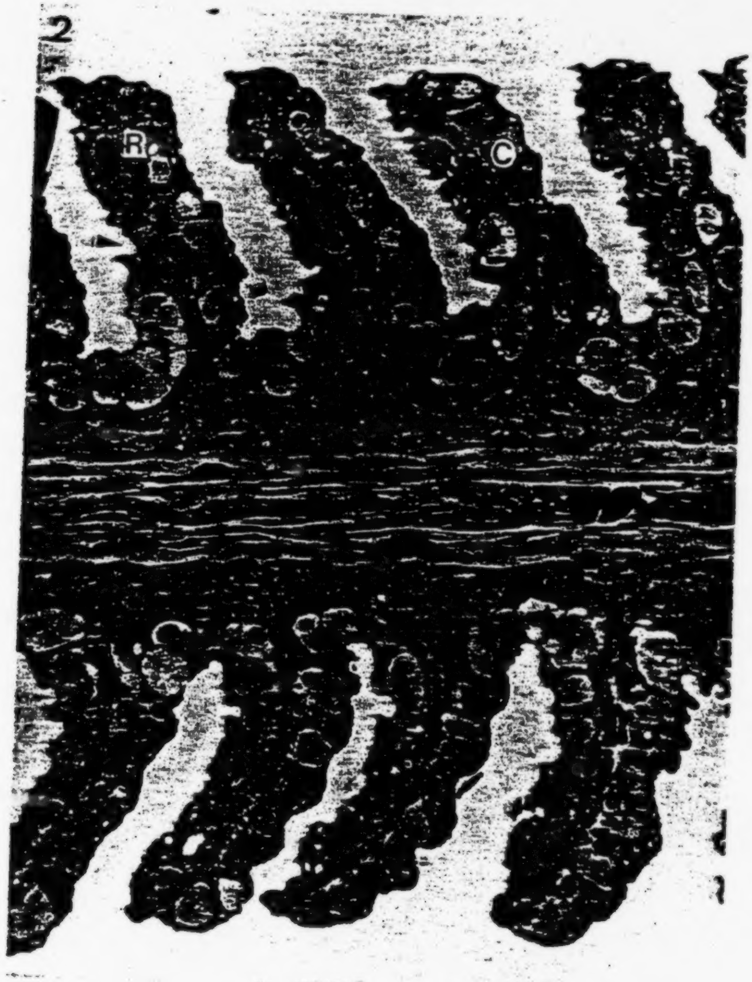


Figure 3. Section of adult rock bass gill from the reference basin, June, 1990, pH 6.1, shows no pathology. Note the thin epithelium of the respiratory lamellae. 314x

3

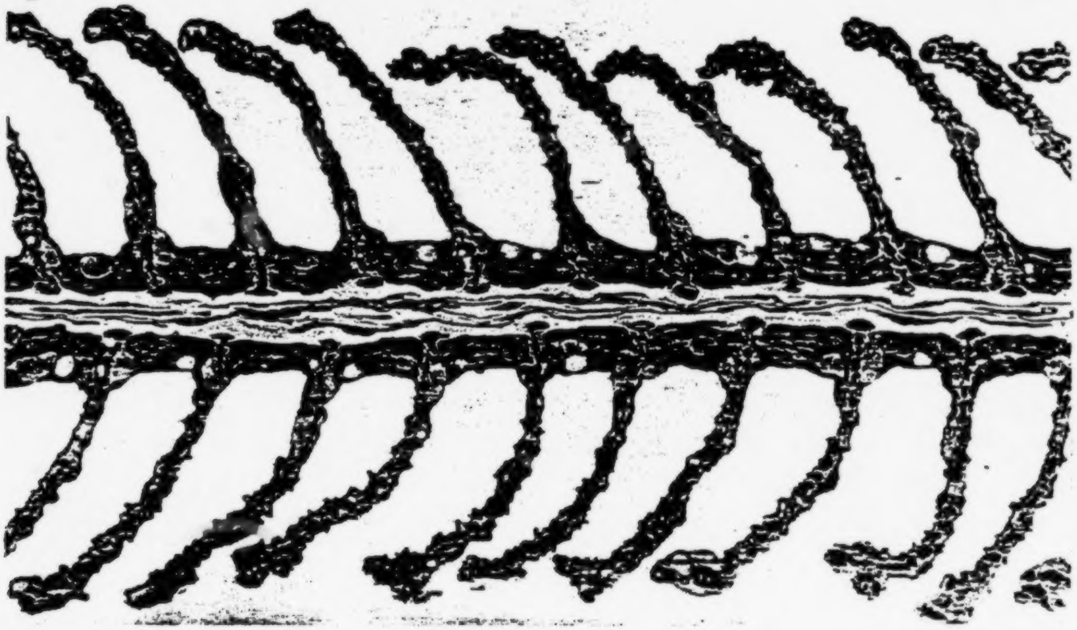


Figure 4. Section of adult rock bass gill from the treatment basin, June, 1990, pH 4.7, showing thickened epithelium, particularly at the bases of the respiratory lamellae (arrowheads), giving the lamellae a triangular appearance. 314x

4

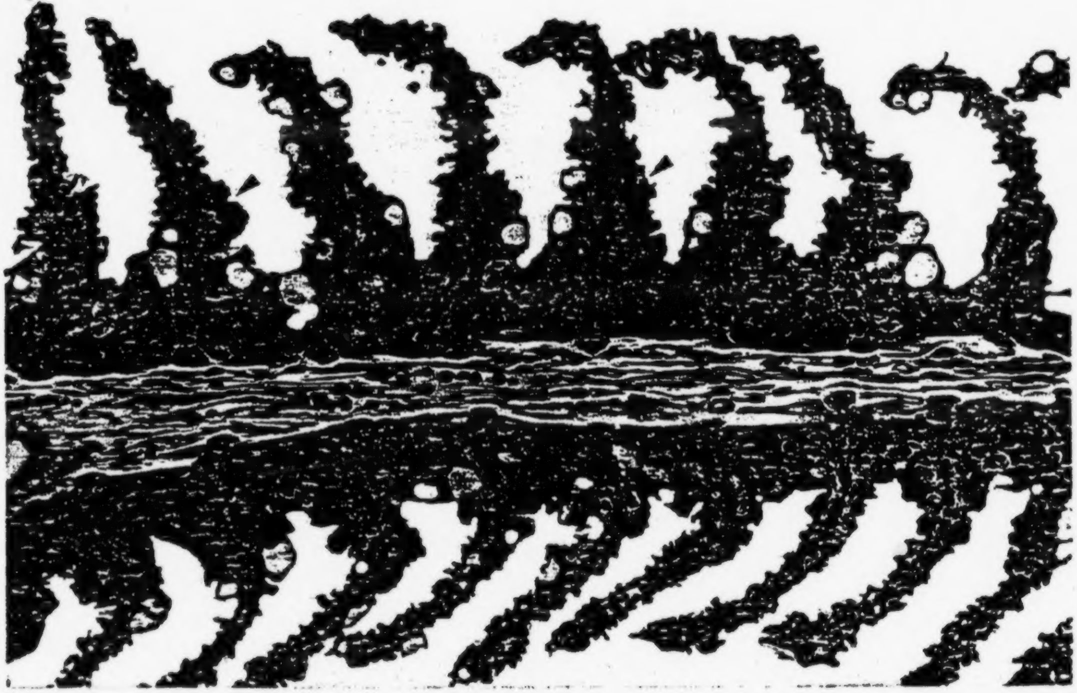


Figure 5. Section of juvenile perch gill from the reference basin, Sept., 1989, pH 6.1, shows no pathology. 475x

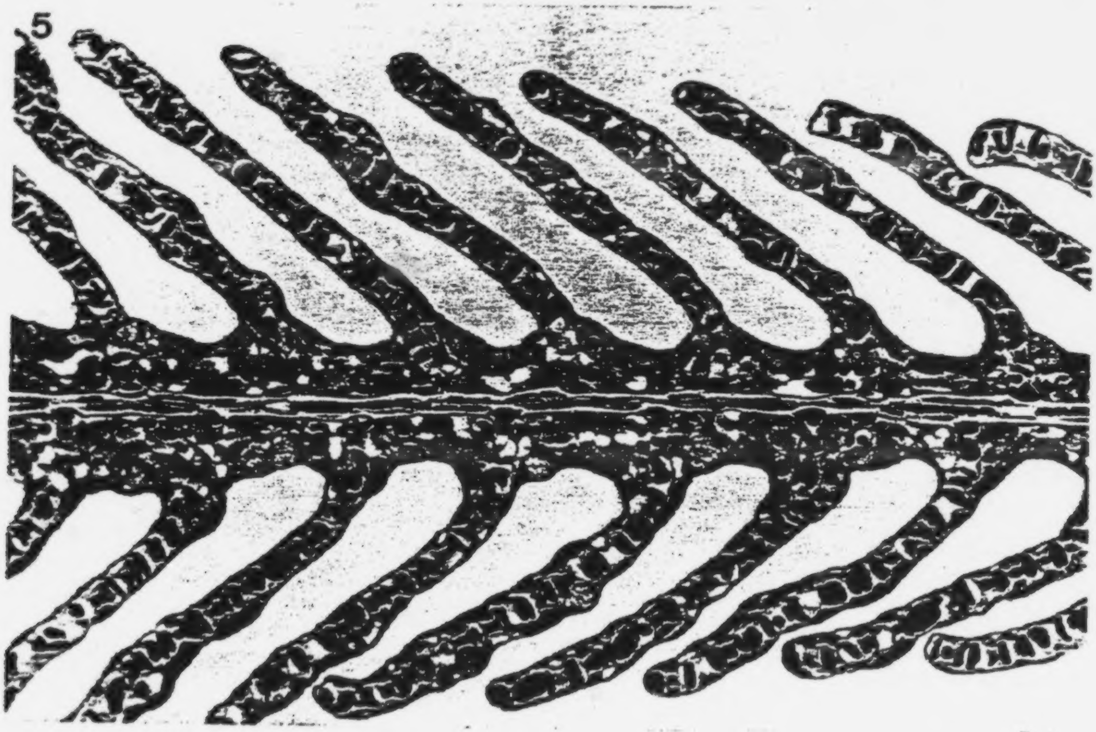
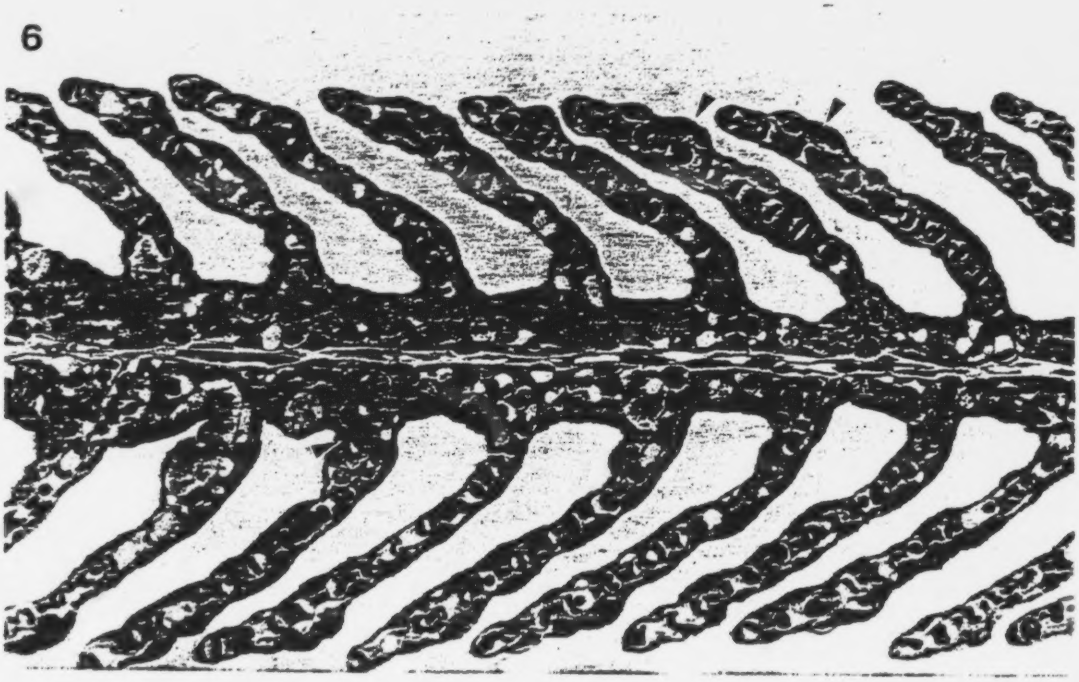


Figure 6. Section of juvenile perch gill from the treatment basin, Sept., 1989, pH 4.7, shows near-normal structure except for proliferated chloride cells (arrowheads). 475x

6



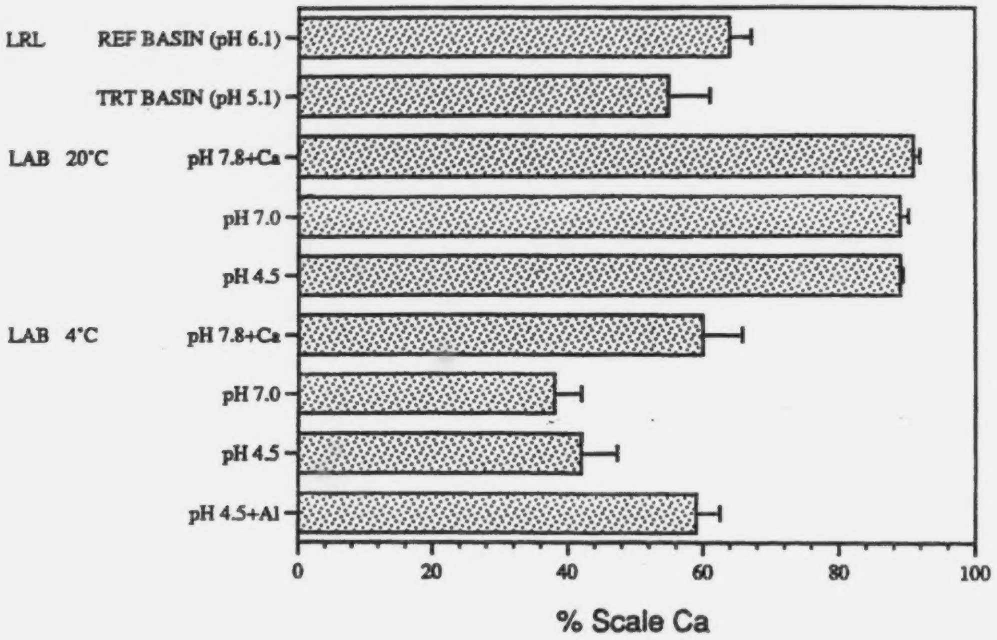


Figure 7. Mean % of scale areas showing positive staining for Ca in juvenile largemouth bass from LRL (9/88 samples) and laboratory exposures. Note high Ca levels in laboratory fish exposed at similar temperatures (20°C) to LRL and low Ca levels in fish exposed to low temperatures stress for 84 days (Leino and McCormick 1993).

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ADAPTATION OF THE INVERTEBRATE COMMUNITY INDEX (ICI) TO THE BENTHOS OF THE ST. LAWRENCE RIVER.

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ABSTRACT

Ohio-EPA's Invertebrate Community Index (ICI) was adapted to the fauna of the St. Lawrence River. Nine of the ten original metrics as well as 6 others were calibrated using published data. Relations between the ICI, metrics and sediment contaminants from the Lake Saint Louis area are considered. The choice of an optimal benthic biotic integrity index based on a subset of metrics composing multimetric indices is discussed.

RÉSUMÉ

L'indice des communautés d'invertébrés, ou ICI, mis au point par le Ohio-EPA a été adapté à la faune du fleuve Saint-Laurent après calibration des métriques en fonction de données historiques publiées. D'autres métriques ont aussi été considérées. Une analyse des relations entre les indices, les métriques et les contaminants du sédiment du lac Saint-Louis a été effectuée. L'optimalisation du choix d'un nombre réduit de métriques composant un indice complexe est discutée.

INTRODUCTION

One of the goals of the Centre Saint-Laurent (CSL) is to develop environmental tools based on biocriteria in order to assess the impact of deleterious factors on ecosystems of the St. Lawrence River. In their review of techniques based on the ecology and ecotoxicology of biological communities, Langlois and Lapierre (1990) mention that the study of benthic community structure is a widely recognized tool for measuring ecosystem health. The present work follows up on the CSL's interest for macroinvertebrate community-related studies and details the adaptation to the St. Lawrence macrozoobenthos, of an indice implemented by the Ohio-EPA (1988a; see also Ohio-EPA, 1987 and 1988b): the Invertebrate Community Index (ICI). Once developed, the St. Lawrence ICI (SL ICI) will be used in programmes such as long-term ecosystem health surveys, or upstream-downstream comparisons for detecting effects of tributaries or effluents.

The ICI corresponds to the cumulated information given by each selected metric composing the indice and illustrates (benthic) biotic integrity (Karr, 1981). Each metric represents a community structure variable known to fluctuate when the benthic assemblage is under the influence of deleterious factors (Ohio-EPA, 1988a; Hellawell, 1989). These components are based on either number of taxa or percent relative abundance of selected faunal groups as well as of pollution tolerant organisms (Table 1).

The first step in the SL ICI development process, the subject of this article, is the calibration of the indice according to the range (and distribution) of values that characterize each selected community structure component representative of the macroinvertebrates in the St. Lawrence River. Also, an analysis of historical sediment contamination data enables us to assess optimal relations between indice variants and abiotic data.

To our knowledge, although ICI or related indices are being adapted in different regions of the USA (see description of EPA's Rapid Bioassessment Protocols in Plafkin et al., 1989 and metric evaluations in Barbour et al., 1992), this adaptation is the first attempt concerning large river biota. Field studies testing the application of the SL ICI method in the St. Lawrence River (Lake Saint Francis, Quebec) have already been published (Pinel-Alloul et al., 1991).

MATERIALS AND METHODS

2.1 Metric calibration

In order to apply the ICI method in a given region, one must establish a calibration scale to determine metric scores from metric values. Ohio-EPA's method takes into account the size of the drainage basin as well as the range of values in reference stations located in ecoregions (Ohio-EPA, 1988a). The St. Lawrence (SL) method is based only on variable range since we are dealing with a large river and not varying hydrological unit sizes. The calibration scale for each metric composing the SL ICI is based on benthic data from 117 stations studied in the Montreal area (Lake of Two Mountains, Lake Saint Louis, Laprairie Basins, Boucherville, Rivière des Prairies, Rivière des Mille-Iles) sampled during 12 surveys between April 1982 and February 1983 (Archipel Project: Ferraris, 1984). The total number of samples is 987. The rationale behind the use of all the samples, as opposed to a fixed space data subset or a fixed temporal subset, is to obtain the widest possible range of metric values.

The stations were selected by Ferraris (1984) in such a manner as to represent the different water masses present: brown water from the Outaouais River, green water from the Great Lakes, and mixed water areas located where the two former masses converge (Figure 1, inset). Other discriminating environmental factors considered by Ferraris (1984) are: presence or absence of macrophytes, current velocity, depth, substrate type, dominant sediment texture.

The calibration graphs (Figure 2; Figures F1 to F4, annex) indicate the cumulative relative number of samples (percent) for an increasing metric value inside the observed range (number of taxa, relative abundance).

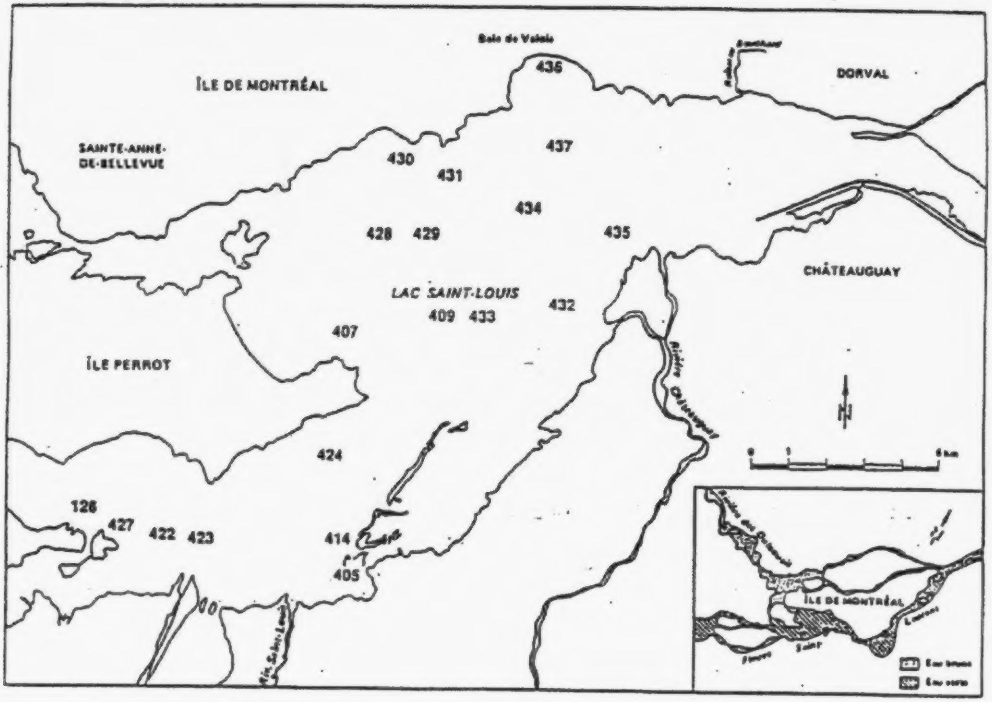

Baie de Valois
436
ÎLE DE MONTRÉAL
DORVAL
SAINTE-ANNE-DE-BELLEVUE
437
430
431
434
428
429
435
CHÂTEAUGUAY
LAC SAINT-LOUIS
409
433
432
407
ÎLE PERROT
Rivière Châteauguay
424
126
427
422
423
414
405
ÎLE DE MONTRÉAL
Saint
Laurent
Eau brune
Eau verte

Figure 1. Localization of Archipel soft bottom macroinvertebrate sites in Lake Saint Louis (Lac Saint-Louis), Quebec. Inset: water masses flowing into Lake Saint Louis: eau brune: brown water (Outaouais River); eau verte: green water (St. Lawrence River: fleuve Saint-Laurent).

Table 1. ST. LAWRENCE (SL) AND OHIO-EPA ICI METRICS.

METRIC	SL ICI	OHIO-EPA ICI
M1	Total number of taxa	
M2	Number of taxa - ephemeroptera	
M3	Number of taxa - trichoptera	
M4	Number of taxa - diptera	
M5	Percent relative abundance - ephemeroptera	
M6	Percent relative abundance - trichoptera	
M7	Percent relative abundance - tanytarsini	
M8	Percent relative abundance - other diptera and non-insect invertebrates	
M9	Percent relative abundance - tolerant taxa (Adapted to SL Benthos: M9A)	Percent relative abundance - tolerant taxa
M10		Percent relative abundance - ephemeroptera, plecoptera and trichoptera

INDEX	ICI (9a)	OHIO-EPA ICI
	Sum of scores from metrics M1 to M9A	Sum of scores from metrics M1 to M10

SUPPLEMENT METRIC	SL ICI	OHIO-EPA ICI
M11	Number of taxa - gasteropoda	-
M12	Percent relative abundance - gasteropoda	-
M13	Number of taxa - oligochaeta	-
M14	Percent relative abundance - oligochaeta	-
M15	Number of taxa - hirudinea	-
M16	Percent relative abundance - hirudinea	-

The graph is subsequently quadrisected via the y-axis and a score of 0, 2, 4 or 6 is allotted to each of the x-axis intervals, keeping in mind the following type of relation associating the score scale and the metric value scale. Eight metrics (metrics M1 to M7 and Ohio-EPA's metric M10) are known to increase in general when perturbation intensity decreases (along a spatial or a temporal scale).

For metrics M8 and M9, a decrease in the latter generally leads to a decrease in the former (Ohio-EPA, 1988a). In the first case, metric scores will increase (0, 2, 4 or 6) to numerically illustrate better biotic integrity at the metric level. In the second case, higher metric scores will be associated with lower metric values. Quadrisection of the graph implies that cumulative sample number frequencies do not necessarily intercept the y-axis for values: 25%, 50% and 75%, but do so for values defined according to the relation:

$$i * \frac{(100 - F\ min)}{4}$$; with i=1, 2 and 3 respectively, and

F min = cumulative percent number of samples (cumulative sample number frequencies) for 0 taxa or 0% relative abundance).

This step leads to a broader 0 metric score interval on the x-axis. On graphs illustrating metrics based on number of taxa, when the first intercept (i=1) is allotted to 0 number of taxa, the next line is adjusted to the next number of taxa encountered; in other cases, the intercept line is moved to the closest number of taxa on the right. For graphs based on percent relative abundance, the vertical lines are fitted to the closest (one) percent unit on the left. Also, the lower limit value has been arbitrarily included in the metric intervals defined by two extreme. Table A1 (see annex) indicates the threshold values obtained after quadrisection and the corresponding metric score values, for each metric calibrated. Figure 2 illustrates the calibration graph for metric M1. All other graphs are placed in the annex (Figures F1 to F4).

2.2 Selection of the list of pollution tolerant taxa composing metric M9

Two definitions of metric M9 (M9A and M9B) are compared in order to choose the most informative version. Other than taxa common to both, metric M9A considers whole taxonomic families: (*Tubificid Oligochaetes* and *Chironomidae Chironomini*) whereas metric 9B only considers particular taxa from these same groups (Table 2).

Table 2. List of taxa composing metric variants M9A and M9B of metric M9 based on % relative abundance of pollution tolerant taxa.

<table>
<tr><th colspan="2">METRIC 9: PERCENT POLLUTION TOLERANT TAXA</th></tr>
<tr><td colspan="2">Common group of taxa
Mollusca Gasteropoda: 7 taxa
Mollusca Pelecypoda: 2 taxa
Oligochaeta Naididae: 7 taxa
Oligochaeta Lumbriculidae: 1 taxa
Annelida Hirudinea: 4 taxa
Insecta Ephemeroptera: 1 taxa
Insecta Diptera: Culicidae and Empididae
Insecta Odonata: 1 taxa
Insecta Hemiptera: Gerridae
Crustacea Isopoda: 2 taxa</td></tr>
<tr><td colspan="2">Differing Groups</td></tr>
<tr><td>Metric M9A</td><td>Metric M9B</td></tr>
<tr><td>Insecta Chironomidae Chironominae Chironomini: all taxa</td><td>Insecta Chironomini: 5 taxa</td></tr>
<tr><td>Oligochaeta Tubificidae: all taxa</td><td>Oligochaeta Tubificidae: 11 taxa</td></tr>
</table>

2.3 Analysis of the relative importance of metrics

In order to assess the relative importance of each defined metric, a macroinvertebrate data set from 19 soft bottom stations located in the Lake Saint Louis area, sampled in July (1982 and 1983) was extracted from the original Archipel Project data set for further analysis. July samples were selected because the ICI(9A) for green water stations, to which are affiliated 17 of the 19 stations considered, show maximal values at this time.

Firstly, a study of the relative importance of each defined metric was conducted by means of a multiple correspondence analysis of the 19 station x 15 metric score classes (metrics M1 to M15; metric M9A, not metric M9B: see discussion). Each metric was defined as a disjunctive variable with four classes (score classes 0, 2, 4 and 6). The metric data set (Table 5) was analyzed by SPAD.N (Lebart et al., 1988). The absolute contribution (Lebart et al., 1982) of each metric, all classes combined, on factorial axis 1 are used to rank the metrics by order of decreasing contribution to the variability expressed by that axis. Secondly, the factorial projections of each metric score class on axis 1 are considered in order to determine the existence of what could be interpreted as environmental gradients expressed at the metric level.

2.4 Correlations between environmental data and metrics or biotic indices

What does an indice such as the ICI, or a variant of the former express when confronted with environmental abiotic data? The contaminant distribution charts obtained from Champoux and Sloterdijk (1988) were employed in order to allot to each selected Lake Saint Louis station (Figure 1), the corresponding value for each environmental variable considered (Table 3). This value is also expressed in disjunctive form. Table 4 indicates the range for each abiotic variable considered and the class value obtained for each station.

Kendall rank correlations were calculated between each environmental variable and metric as well as with the ICI(9A) and the sum of the scores from all 15 metrics, labelled I16 (Table 6). Since the number of stations considered was between 10 and 30 (n=19), a correction was applied to the Kendall coefficients (calculation of Z_τ) for subsequent significance evaluation in the $\Phi(z)$ tables (Scherrer 1984, p. 608). After each iterative addition of metric scores ranked by decreasing value of absolute contribution, Kendall correlations were calculated in order to establish the most significantly indicative metric combination (Tables 7, 8-a and 8-b).

RESULTS AND DISCUSSION

Low ICI values indicate strongly perturbed benthic communities therefore diminished biotic integrity. An increase in the ICI is indicative of better biotic integrity and of benthic community structure more typical of lesser perturbed aquatic environments. Reference sites (usually chosen in the least impacted areas) generally give highest ICI values for a given region (Ohio-EPA, 1988a).

The ICI is calculated by simply summing metric scores. Ohio-EPA's ICI varies between 0 and 60, whereas the SL ICI, ICI(9A), varies between 0 and 54 as it is based only on 9 metrics (Table 1). Another SL ICI, called I16, is also tested here and varies between 0 and (15x6 =) 90. The difference between the 9 metric SL ICI and the Ohio-EPA ICI is due to sampling methodology employed. The Ohio-EPA uses artificial substrates to collect benthic fauna whereas SL incites are based on fauna collected on natural substrates. The Ohio ICI does consider data from bottom samples to assess the influence of habitat structure, as expressed through metric M10 (Table 1).

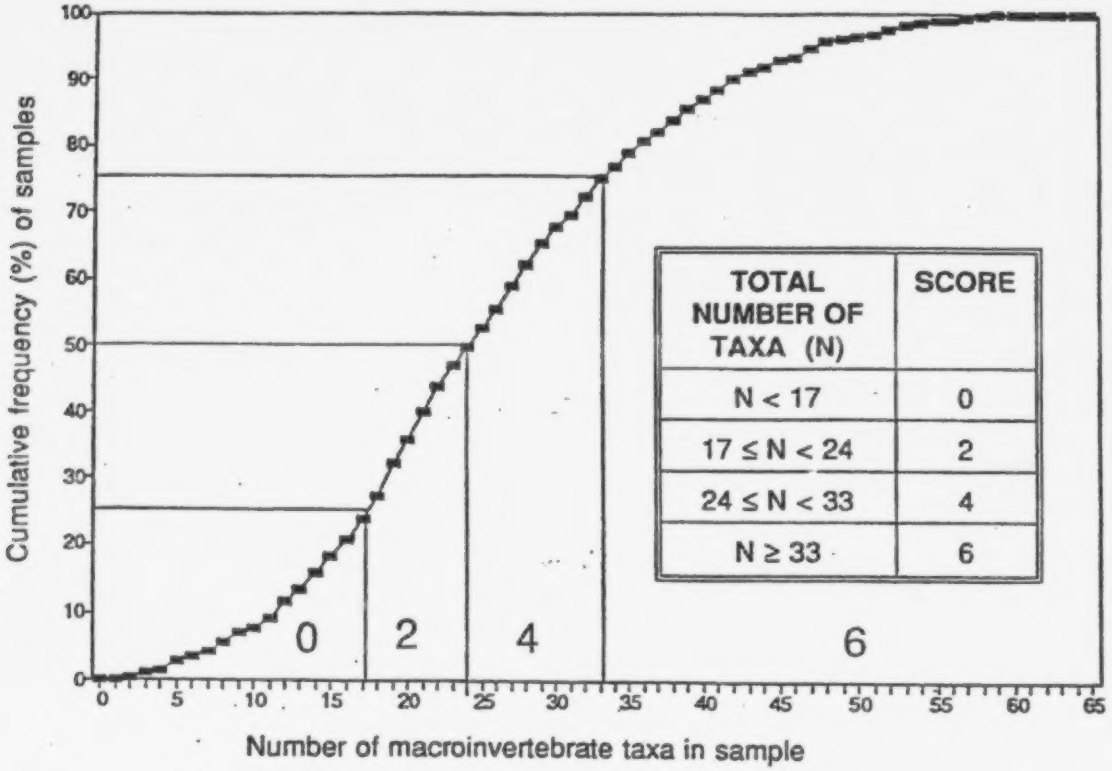
Contrarily to the Ohio-EPA, the CSL suggests sampling the natural substrate in order to include the influence of habitat structure in all metrics considered, which makes the consideration of Ohio-EPA's metric M10 unnecessary.

3.1 Calibration graphs

Figure 2 shows the calibration curve for metric M1, the total number of macroinvertebrate taxa. The thresholds and scores for each defined class are indicated in Table A1 (see annex) along with graphs for the other metrics (Figures A1 to A4). Once obtained, raw data (metric values) from each of the station samples are transformed into scores. This process assures the local adaptation of metrics defined for uses in other areas whether they be those discussed in this paper or applied to other metrics studied in the future (Ohio-EPA, 1988a, Barbour et al., 1992).

3.2 Variants M9A and M9B

Nine metric ICI values calculated on the one hand with metric M9A and the other hand with metric M9B (Table 2) indicate similar spatial and temporal variations. Since metric M9A considers faunal groups at a higher taxonomic level, this metric was judged more practical to use, therefore metric M9B was eliminated at this point. ICI values are thus indicated as ICI(9A) in order to avoid confusion with the Ohio-EPA version or future variants of the St. Lawrence indice.



TOTAL NUMBER OF TAXA (N)	SCORE
N < 17	0
17 ≤ N < 24	2
24 ≤ N < 33	4
N ≥ 33	6

Figure 2. Calibration graph for metric M1: total number of macroinvertebrate taxa. See annex for calibration graphs for other metrics.

3.3 Relative importance of each metric

The three first factorial axes obtained by the multiple correspondence analysis extract a total of 41,1% of the variance expressed by the biotic matrix analyzed (axis 1: 16,4%; axis 2: 12,5; axis 3: 12,2%). This low variance value is not surprising since the benthic data, notorious for low variance extraction when factorial techniques are employed, have been previously transformed, which always leads to a loss of information. A factorial analysis based on the taxon density data set could have been conducted, but the rationale here is to exploit the score transformed data.

Table 6 indicates the ranked metrics according to decreasing absolute contribution to axis 1 values. Top ranking metrics include some composing the ICI(9A) such as metrics M6, M9A and M8, as well as complementary metrics such as M13 and M14. It is interesting to note that certain ICI(9A) metrics such as metrics M4 and M2 do not contribute greatly to the variance expressed by axis 1. The low absolute contribution of metric M2, which refers to the number of taxa of the Ephemeroptera Insect group, seems to indicate in this context that this group does not bring a considerable amount of information to the ICI(9A). Whether this is a situation generalizable to all of the St. Lawrence biota remains to be assessed. Nevertheless, these results illustrate the need for adaptation to local fauna of the constituents of a biotic indice such as the ICI.

The interpretation of the environmental meaning of factorial axes is greatly helped by the use of supplement abiotic variables in multivariate analyses (Lebart et al., 1982). The factorial projection of these variables on a given axis are classically employed to characterize the gradients or more often opposition between station groups along a given axis. In this case, since the analysis is based on the use of biotic variables expressed as classes, the projections of the latter on axis 1 may themselves suggest the existence of a biotic gradient, reflecting the gradual influence of environmental conditions. Here, we have metric classes defined in such a manner as to express increasing (partial) biotic integrity as the score increases from 0 to 6.

Thus, along factorial axis 1, certain metrics show distinct opposition of low scoring classes and high scoring classes (Table 6). The non-existence of certain score classes are due to their non representation in the data matrix (Table 5). The position of the score values in Table 6 reflect their relative positions to the axis origin. Although only the fifth highest ranking metric by absolute contribution to axis 1, metric M14 indicates a "perfect" gradient with score class values 0, 2, 4 and 6 positioned in this order in the direction of the negative extremity of axis 1. The negative portion of axis 1 is characterized by high scoring metric classes. Conversely, metrics M1, M5, M11, M4 and M2 show higher score classes on the positive portion of the same axis.

Table 3. Value ranges (min: minimum; max: maximum) and corresponding classes (class) of abiotic variables analyzed by Champoux and Sloterdijk (1988).

VARIABLE		RANGES AND CLASSES						
AL	silt-clay fraction (% weight)	min	0,00	16,67	33,33	50,00	66,67	83,33
		max	16,67	33,33	50,00	66,67	83,33	100,00
		class	1	2	3	4	5	6
EM	Major elements: Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn, P (% mg.kg^{-1})	min	1,00	1,80	2,60	3,40	4,20	
		max	1,80	2,60	3,40	4,20	5,00	
		class	1	2	3	4	5	
CO	organic carbon (%mg.kg^{-1})	min	0,38	1,72	3,06	4,41	5,75	7,09
		max	1,72	3,06	4,41	5,75	7,09	8,43
		class	1	2	3	4	5	6
NT	total nitrogen (% mg.kg^{-1})	min	0,00	0,10	0,20	0,30	0,40	0,50
		max	0,10	0,20	0,30	0,40	0,50	0,60
		class	1	2	3	4	5	6
As	arsenic (mg.kg^{-1})	min	0,00	4,23	8,45	12,68	16,90	21,13
		max	4,23	8,45	12,68	16,90	21,13	25,35
		class	1	2	3	4	5	6
Cd	cadmium (mg.kg^{-1})	min	0,00	0,47	0,93	1,40	1,87	2,33
		max	0,47	0,93	1,40	1,87	2,33	2,80
		class	1	2	3	4	5	6
Cr	chromium (mg.kg^{-1})	min	39,00	62,33	85,67	109,00	132,33	155,67
		max	62,33	85,67	109,00	132,33	155,67	179,00
		class	1	2	3	4	5	6
Cu	copper (mg.kg^{-1})	min	7,00	17,17	27,33	37,50	47,67	57,83
		max	17,17	27,33	37,50	47,67	57,83	68,00
		class	1	2	3	4	5	6
Ni	nickel (mg.kg^{-1})	min	8,00	18,17	28,33	38,50	48,67	58,83
		max	18,17	28,33	38,50	48,67	58,83	69,00
		class	1	2	3	4	5	6

Table 3. (cont'd) - Value ranges (min: minimum; max: maximum) and corresponding classes (class) of abiotic variables analyzed by Champoux and Sloterdijk (1988).

VARIABLE		RANGES AND CLASSES						
Pb	lead ($mg.kg^{-1}$)	min	11,00	26,00	41,00	56,00	71,00	86,00
		max	26,00	41,00	56,00	71,00	86,00	101,00
		class	1	2	3	4	5	6
Zn	zinc ($mg.kg^{-1}$)	min	19,00	130,17	241,33	352,50	463,67	574,83
		max	130,17	241,33	352,50	463,67	574,83	686,00
		class	1	2	3	4	5	6
DDE	pp'DDE ($mg.kg^{-1}$)	min	0,000	0,002	0,004	0,006		
		max	0,002	0,004	0,006	0,008		
		class	1	2	3	4		
BPC	total PCB's ($mg.kg^{-1}$)	min	0,00	0,10	0,19			
		max	0,10	0,19	0,29			
		class	1	2	3			
MIR	Mirex (10^{-2} $mg.kg^{-1}$)	min	0,00	0,02	0,05	0,07	0,09	0,11
		max	0,02	0,05	0,07	0,09	0,11	0,14
		class	1	2	3	4	5	6
HAP	PAH ($mg.kg^{-1}$)	min	0,03	0,65	1,26	1,88	2,49	3,11
		max	0,65	1,26	1,88	2,49	3,11	3,72
		class	1	2	3	4	5	6
IC	contamination index	min	0,39	0,93	1,48	2,02	2,56	3,11
		max	0,93	1,48	2,02	2,56	3,11	3,65
		class	1	2	3	4	5	6

3.4 Relations between environmental variables and macroinvertebrate community structure

3.4.1 Relations between individual metrics and abiotic variables

If metric score variations are indicative of improving environmental conditions when scores increase from 0 to 6, correlations with potentially deleterious abiotic variables (such as heavy metals or organic contaminants) in sediments should be negative. Alternate hypotheses may be formulated. Firstly, the concentrations of contaminants in the sediment show variations that are interpreted by means of covarying non (or lesser) deleterious factors. Secondly, the abiotic as well as biotic data ranges show too much discrepancy and therefore significant statistical results are difficult to interpret objectively.

Table 4. Lake Saint Louis abiotic variables measured in superficial sediment samples: value classes.

STATION	VALUE CLASSES															
	AL	EM	CO	NT	As	Cd	Cr	Cu	Ni	Pb	Zn	DDE	BPC	MIR	HAP	IC
126	6	3	3	1	1	4	4	4	5	4	3	2	1	1	1	4
405	5	2	4	5	2	2	2	4	3	1	2	1	2	5	2	3
407	2	1	1	1	1	4	1	1	1	1	1	1	1	1	1	2
409	2	2	1	1	1	0	5	1	1	1	2	1	1	1	3	2
414	5	2	3	4	2	2	2	3	3	1	2	1	2	4	3	3
422	5	3	3	4	2	4	4	6	5	4	4	2	1	3	1	5
423	3	3	3	4	2	3	4	6	5	4	4	2	1	4	1	4
424	2	2	1	1	1	2	3	1	2	1	1	1	1	1	2	1
427	6	3	3	2	1	4	4	5	5	4	4	2	1	1	1	5
428	5	2	2	3	1	6	4	3	2	3	2	2	2	2	3	4
429	5	2	2	2	1	6	4	2	2	1	1	2	2	1	2	3
430	5	3	3	3	2	6	4	5	4	4	4	1	2	2	2	5
431	6	2	3	3	2	6	2	5	4	3	4	4	3	4	2	5
432	2	2	2	1	1	2	4	2	2	1	1	1	1	1	2	2
433	2	2	1	1	1	2	4	1	1	1	1	1	1	1	3	2
434	5	2	2	4	1	4	2	3	4	2	3	2	3	4	2	3
435	1	1	1	4	1	1	5	1	1	1	1	1	1	1	1	1
436	4	4	1	2	1	1	5	2	1	2	2	1	1	1	6	1
437	2	4	2	2	5	3	4	3	5	3	4	1	1	1	2	4

Metric M12 does not show any significant correlations with any abiotic variable (Table 7). Four of these variables do not significantly correlate with any of the defined metrics: percentage of combined silt and clay fraction (AL), percent organic carbon (CO), PAH (HAP) concentrations and the contaminant index (IC) calculated by Champoux and Sloterdijk (1988). Metrics showing only negative correlations (Table 7) are: M4, M7, M13, M14 and M16, two of which (M4 and M7) contribute to the expression of the ICI(9A).

Metric M4, based on the number of Diptera taxa, is negatively correlated with the sediment chromium (Cr) concentration whereas metric M7 based on the relative abundance of Tanytarsini is negatively correlated with sediment concentrations of total nitrogen (NT), total PCB (BPC) and Mirex (MIR).

Table 5. Lake Saint Louis stations score data set and indice for ICI(9A) and I16.

STA-TIONS	METRIC SCORES															SL INDICES	
	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M9 (A)	M 11	M 12	M 13	M 14	M 15	M 16	ICI (9A)	I16
126	6	2	6	4	0	2	0	0	2	6	2	0	2	2	6	22	40
405	6	0	4	6	0	0	0	0	2	6	6	2	4	0	2	18	38
407	6	0	0	6	0	0	2	0	0	4	2	0	2	0	2	14	24
409	4	0	6	6	0	6	6	2	4	4	0	2	6	4	4	34	54
414	6	0	6	6	0	0	0	0	0	6	2	4	0	0	6	18	36
422	4	2	2	4	0	0	0	0	0	6	0	0	0	0	0	12	18
423	6	2	6	6	0	0	0	0	0	4	0	0	0	4	0	20	28
424	6	2	4	6	0	0	0	0	0	6	4	0	2	0	2	18	32
427	4	2	0	6	6	0	2	0	2	6	2	0	0	6	6	22	42
428	4	0	4	4	0	2	0	0	0	4	4	0	2	2	2	14	28
429	4	2	6	4	0	2	0	0	0	4	4	2	4	4	4	18	40
430	6	2	6	4	2	6	0	4	0	6	6	2	4	4	6	30	58
431	4	2	6	4	0	2	0	0	0	4	4	2	4	2	2	18	36
432	4	0	2	4	0	0	0	0	4	6	4	2	4	4	6	14	40
433	6	0	4	6	0	0	0	0	0	6	6	0	4	4	6	18	42
434	6	0	6	6	0	0	0	0	0	6	6	0	6	0	4	18	40
435	2	0	4	4	0	6	0	4	6	4	6	6	6	4	6	26	58
436	4	4	4	4	2	2	0	0	2	6	4	2	2	4	6	22	46
437	6	4	6	6	2	4	2	0	0	6	6	4	4	4	6	30	60

Champoux and Sloterdijk (1988) conclude that superficial sediment Cr is associated with the sand fraction which they interpret as a result of a lithological influence on that metal's distribution. In this case, it is difficult to assess if the concentration of sediment Cr may be more indicative of a toxic effect on the Diptera or if it illustrates the presence of a sediment type less convenient for colonization by taxa of this group. According to Champoux and Sloterdijk (1988), the source, modes of transportation and sedimentation of the total PCEs (BPC) and Mirex (MIR) are similar. These authors have shown that the two contaminants are not significantly correlated with the sediment percent content of clay nor with that of the combined silt-clay fraction.

Table 6. Ranked metrics by decreasing absolute contributions to axis 1. Position and order of the projection on axis 1 (negative portion: -; positive portion: +) of each metric score class.

RANKED METRICS	ABSOLUTE CONTRIBUTION OF METRIC (ALL CLASSES) TO AXIS 1	ORDER OF PROJECTION OF METRIC SCORE CLASS ON:	
		AXIS 1 -	AXIS 1 +
M6: % TRICHOPTERA	11,5 %	6	2 ; 4 ; 0
M13: T. OLIGOCHAETA	11,3 %	6 ; 2	4 ; 0
M9A: % TOLERANT TAXA	11,0 %	6 ; 4	0 ; 2
M8: % O. DIPT. + NON INSECTS	10,8 %	2 ; 4	0
M14: % OLIGOCHAETA	10,6 %	6 ; 4	2 ; 0
M15: T. HIRUDINEA	7,9 %	4	2 ; 0 ; 6
M1: TOTAL TAXA	7,5 %	2 ; 4	6
M7: % TANYTARSINI	6,6 %	6 ; 0	2
M12: % GASTEROPODA	5,8 %	6 ; 0 ; 4	2
M3: T. TRICHOPTERA	4,6 %	4 ; 6	2 ; 0
M5: % EPHEMEROPTERA	3,4 %	2 ; 0	6
M16: % HIRUDINEA	2,8 %	4 ; 6	2 ; 0
M11: T. GASTEROPODA	2,7 %	2	6
M4: T. DIPTERA	2,0 %	4	6
M2: T. EPHEMEROPTERA	1,7 %	0	4 ; 2

They do indicate however a strong positive correlation between sediment (particulate) organic matter and respectively: BPC, MIR and pp'DDE (DDE). This last point is coherent with the existence of an inverse correlation between the number of *Tubificid Oligochaete* taxa (metric M13), that thrive on particulate organic matter (Hellawell, 1989) and pp'DDE concentrations in the sediment of Lake Saint Louis.

Metrics M2, M3, M5 and M6 show positive correlations with several abiotic variables (Table 7). Metrics M2 and M5, defined respectively from the number of taxa and relative abundance of Ephemeroptera, are positively correlated with the major elements (EM) (composed of: Si, Al, Fe, Mg, Ca, Na, K, Ti, Mn and P; see Champoux and Sloterdijk, 1988) found in the superficial sediments.

The stations representative of green water (St. Lawrence River water originating from the Great Lakes) are mainly grouped in the EM class 2 which is in accordance with conclusions formulated by Champoux and Sloterdijk (1988). The EM class 3, according to the same authors, represents stations influenced by the Outaouais River (brown water).

Table 7. Kendall rank correlations between metrics scores, indices (ICI(9A) and I16) and abiotic variables (for n = 19, the correlation coefficient must be equal or greater than 0,333 to be significant at or smaller than alpha = 0,05).

ABIOTIC VARIABLES	METRICS									SL INDICE
	M1	M2	M3	M4	M5	M6	M7	M8	M9A	ICI (9A)
AL	#	#	#	#	#	#	#	#	#	#
EM	#	0,773	#	#	0,614	#	#	#	#	0,345
CO	#	#	#	#	#	#	#	#	#	#
NT	#	#	#	#	#	#	- 0,366	#	#	#
As	0,309	#	0,330	#	#	#	#	#	- 0,362	#
Cd	#	#	#	#	#	#	#	#	- 0,507	#
Cr	- 0,523	#	#	- 0,361	#	0,522	#	0,445	0,476	0,409
Cu	#	0,344	#	#	#	#	#	#	#	#
Ni	#	0,442	#	#	#	#	#	#	#	#
Pb	#	0,494	#	#	0,424	#	#	#	#	#
Zn	#	0,460	#	#	0,420	#	#	#	#	#
DDE	#	#	#	#	#	#	#	- 0,348	#	#
BPC	#	#	0,418	#	#	#	- 0,364	#	- 0,365	#
MIR	#	#	#	#	#	#	- 0,386	#	#	#
HAP	#	#	#	#	#	#	#	#	#	#
IC	#	#	#	#	#	#	#	#	#	#

Table 7. (cont'd) - Kendall rank correlations between metrics scores, indices (ICI(9A) and I16) and abiotic variables (for n = 19, the correlation coefficient must be equal or greater than 0,333 to be significant at or smaller than alpha = 0,05).

ABIOTIC VARIABLES	METRICS						SL INDICE
	M11	M12	M13	M14	M15	M16	I16
AL	#	#	#	#	#	#	#
EM	0,450	#	#	- 0,336	#	#	#
CO	#	#	#	#	#	#	#
NT	#	#	#	#	#	#	#
As	#	#	#	#	#	#	#
Cd	#	#	#	#	#	#	#
Cr	#	#	#	#	0,641	#	0,459
Cu	#	#	#	- 0,360	#	#	#
Ni	#	#	#	#	#	#	#
Pb	#	#	#	- 0,373	#	#	#
Zn	#	#	#	#	#	#	#
DDE	- 0,289	#	- 0,405	#	#	- 0,373	- 0,340
BPC	#	#	#	#	#	#	#
MIR	#	#	#	#	- 0,460	- 0,418	#
HAP	#	#	#	#	#	#	#
IC	#	#	#	#	#	#	#

We may conclude therefore that our results clearly show that the EM classes indicate a shift in water mass type influencing the benthos. Ferraris (1984) has demonstrated the major influence of water mass type on the definition of benthic macroinvertebrate community structure and has stated that the Ephemeroptera are more abundant in brown and mixed water stations than in green water sites. Surprisingly, metric M2 based on Ephemeroptera taxa is positively correlated with the sediment distribution of four metals: Cu, Ni, Pb and Zn.

The stations representative of green water (St. Lawrence River water originating from the Great Lakes) are mainly grouped in the EM class 2 which is in accordance with conclusions formulated by Champoux and Sloterdijk (1988). The EM class 3, according to the same authors, represents stations influenced by the Outaouais River (brown water).

Champoux and Sloterdijk (1988) have indicated that these four substances show very similar distribution patterns in Lake Saint Louis and that they accumulate in sediment deposition zones. Metric M5 shows positive correlations with only two of these: Pb and Zn. Ferraris (1984) has also demonstrated that the major Ephemeroptera taxa (*Hexagenia* and *Caenis*) are more abundant in deeper stations with low current velocity. The genera *Caenis* is considered as being a pollution tolerant taxon by different authors (Hellawell, 1989; Plafkin et al. 1989) and is included in the list of taxa defining metric M9A.

3.4.2 Relations between the ICI(9A), composing metrics and abiotic variables

Correlations between ICI(9A) and abiotic variables indicate significant positive relations between the major element group (EM) as well as with the sediment concentration in Cr (Table 7). All other significant correlations expressed with individual metrics and the abiotic variables seem to have been occurred after summing scores. If the ICI is an integrating index, these results would therefore demonstrate the presumable major importance of these two abiotic variables on the expression of the macroinvertebrate structure as illustrated by the ICI(9A). It is interesting to note that the two significant correlations between the ICI(9A) and abiotic data pertain to variables indicative of the influence of water mass type (EM and, hypothetically, Cr) on the macroinvertebrate community structure.

There are no metrics which present correlations with the same two abiotic variables correlated with the index ICI(9A) (Table 7). Only metrics M4 and M6 are significantly associated with sediment Cr variations, although opposingly. The resulting influence of variable EM as expressed by the significant correlation with the ICI(9A) is expressed at the metric level by M2 and M5 only, although these metrics correlate with other abiotic variables (Table 7).

3.4.3 Relations between I16 and abiotic variables

If one sums up the scores of all 15 metrics defined, one can calculate another indice, called indice I16 (to illustrate the number of the last metric considered). This index integrates the partial biotic integrity expressed by each of the 15 defined metrics (M9B excluded here as well).

This approach uses the plasticity of the definition of an index such as one obtained through the addition of community structure variables considered as sub-units (metrics). Significant Kendall correlations between I16 and abiotic variables show a positive relation - with sediment Cr, a result comparable with the ICI(9A). I16 is negatively correlated with pp'DDE (DDE), an organic contaminant strongly associated with particulate organic matter (Champoux and Sloterdijk, 1988). Here again one must question whether the indice correlations with the two abiotic variables denote toxic effect (of pp'DDE) or simply illustrate the influence on the macroinvertebrate community structure of the physical strata (sand fraction for Cr, particulate organic matter for pp'DDE) to which the substance is associated. Metrics composing the I16 and illustrating the significant correlation with sediment Cr only are: M4, M6 and M15. The resultant correlation expressed by I16 is positive, whereas M4 indicates a negative correlation with sediment Cr (Table 7). Metrics illustrating the correlation with DDE only are: M13 and M16.

3.5 Selection of an optimal biotic index

Can one define an index with less metrics than the I16 or the ICI(9A) but still obtain the same correlations with abiotic variables? In order to answer this question, one may consider a series of combinations of a smaller number of summed metrics which are then correlated with the abiotic variables. In order to limit these combinations to the most significant ones, a criteria must be adopted with which the metrics can be ranked by decreasing order of importance. In this case, the absolute contributions of the metrics to factorial axis 1 (Table 6) were utilized for this purpose. In a second step, the most contributing metric (metric M6) was correlated with abiotic variables. Then the scores from the first two contributing metrics scores (Metrics IA6 and M13) were added and Kendall correlations calculated once more.

This process was executed in an iterative manner and ended in the summing of the scores of the fifteen metrics, which is the I16. Table 8-a indicates the significant Kendall correlations obtained. It is interesting to point out that after eight iterative additions, a sub-unit of I16, composed of metrics M6+ M13+ M9A+ M8+ M14+ M15+ M1+ M7+ M12, shows significant correlations with the same abiotic variables as the I16. One iterative step, obtained after adding the score of metric M14, leads to no significant correlations (Table 8-a). This seems to indicate that metric M14 does not contribute to the system and should therefore be eliminated.

Following this result, a second set of iterative additions and correlations were conducted (Table 8-b). In this case, one may notice that significant correlations are obtained with both sediment Cr and pp'DDE in a manner similar to those expressed by I16 after only seven iterative additions. These results lead us to believe that an indice based on the information expressed by summed metrics M6, M13, M9A, M8, M15, M1, M7 and M12 is sufficient for the assessment of the correlative associations between both abiotic and biotic data analyzed in this study.

Table 8a. Significant Kendall correlations between iteratively summed metric scores and abiotic variables. The last iteration corresponds to I16. For n = 19, the correlation coefficient must be equal or greater than 0,333 to be significant at or under alpha = 0,05.

ITERATIVE SUMMATION OF RANKED METRIC SCORES	SIGNIFICANTLY CORRELATED ABIOTIC VARIABLES	
	Cr	DDE
M6	0,522	#
M6+13	0,366	#
M6+13+9A	0,448	#
M6+13+9A+8	0,447	#
M6+13+9A+8+14	#	#
M6+13+9A+8+14+15	0,495	#
M6+13+9A+8+14+15+1	0,460	#
M6+13+9A+8+14+15+1+7	0,459	#
M6+13+9A+8+14+15+1+7+12	0,402	- 0,365
M6+13+9A+8+14+15+1+7+12+3	0,362	#
M6+13+9A+8+14+15+1+7+12+3+5	0,416	#
M6+13+9A+8+14+15+1+7+12+3+5+16	0,462	- 0,344
M6+13+9A+8+14+15+1+7+12+3+5+16+11	0,432	- 0,391
M6+13+9A+8+14+15+1+7+12+3+5+16+11+4	0,396	- 0,399
M6+13+9A+8+14+15+1+7+12+3+5+16+11+4+2 = I16	0,459	- 0,340

Table 8-b. Significant Kendall correlations between iteratively summed metric scores and abiotic variables. The last iteration corresponds to I16. For n = 19, the correlation coefficient must be equal or greater than 0,333 to be significant at or under alpha = 0,05.

ITERATIVE SUMMATION OF RANKED METRIC SCORES	SIGNIFICANTLY CORRELATED ABIOTIC VARIABLES		
	Cr	DDE	MIR
M6	0,522	#	#
M6+13	0,366	#	#
M6+13+9A	0,448	#	#
M6+13+9A+8	0,447	#	#
M6+13+9A+8+15	0,607	#	#
M6+13+9A+8+15+1	0,583	#	#
M6+13+9A+8+15+1+7	0,559	#	- 0,340
M6+13+9A+8+15+1+7+12	0,503	- 0,378	#
M6+13+9A+8+15+1+7+12+3	0,453	#	#
M6+13+9A+8+15+1+7+12+3+5	0,502	#	#
M6+13+9A+8+15+1+7+12+3+5+16	0,525	#	#
M6+13+9A+8+15+1+7+12+3+5+16+11	0,491	- 0,369	#
M6+13+9A+8+15+1+7+12+3+5+16+11+4	0,439	- 0,406	#
M6+13+9A+8+15+1+7+12+3+5+16+11+4+2 = I16 - M14	0,452	- 0,358	#

CONCLUSIONS

Natural substrate sampling methods are suggested for macroinvertebrate benthos data because the influence of the biotope will be expressed in each of the metrics defining the ICI. It is thought that the influences on benthic community structure by both the biotope and abiotic environmental factors, deleterious or not, is better assessed in this manner. Although the published results on the use of the ICI seem promising (Ohio EPA, 1987, 1988a, 1988b; Plafkin et al., 1989; Barbour et al., 1992), Ohio EPA's ICI could not be directly applied to the macroinvertebrate fauna of the St. Lawrence river. Calibration graphs permitted the necessary adaptation of the ICI metrics.

In conformity with the Ohio EPA approach, four metric score classes were considered. In the future, the possibility of modifying the number of classes must be exploited in cases such as regional variations in community structure or the establishment of the precision level required for optimal environmental management of biotic integrity. One example pertains to the ICI's predecessor, based on fish community metrics, the IBI (index of biotic integrity), which is based on only three classes: scores 1, 3 and 5 (Karr, 1981).

What metrics should compose an indice such as the ICI? Each metric brings into the ICI its own variability. Results presented in this paper demonstrate the possible loss of information through the summation of opposing abiotic factor-related metrics. Also, some of the supplement metrics defined and studied in this paper proved to be more significant than metrics composing the ICI(9A). Furthermore a series of combined metric summations has shown to indicate the same relations between biotic (transformed) data and abiotic variables as one of the indices tested (I16). This approach globally leads us to conclude that the answer to the question is the following: the definition and use of metrics through a composite indice such as the ICI must be conducted with the finality of the assessment programme in mind. It is utopic to consider that an index developed in one area will be directly applicable in another area, submitted most probably to other types or intensities of environmental stressors acting on an ecosystem differing by its biotope and (or) its biota. Each management programme must commence with an initial phase during which every environmental tool envisaged will be fined-tuned to the required level of response sensitivity. In the long run, the approach, if executed in a pluridisciplinary context, may prove to be cost-effective and therefore assure its continuity through time.

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Table A1. Metric value interval thresholds and corresponding scores obtained after calibration with the benthic data set from Ferraris (1984).

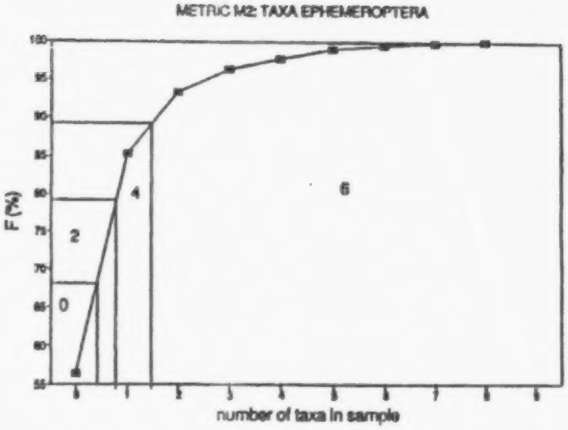
METRICS	THRESHOLD VALUES	SCORES
M1: total number of taxa	N < 17	0
	17 ≤ N < 24	2
	24 ≤ N < 33	4
	N ≥ 33	6
M2: number of taxa - Ephemeroptera	N = 0	0
	N = 1	2
	N = 2	4
	N ≥ 3	6
M3: number of taxa - Trichoptera	N = 0	0
	N = 1	2
	N = 2	4
	N ≥ 3	6
M4: number of taxa - Diptera	N = 0 ou 1	0
	N = 2 ou 3	2
	N = 4 à 7	4
	N ≥ 8	6
M5: percent relative abundance - Ephemeroptera	% < 1	0
	1 ≤ % < 3	2
	3 ≤ % < 4	4
	% ≥ 4	6
M6: percent relative abundance - Trichoptera	% < 1	0
	1 ≤ % < 2	2
	2 ≤ % < 4	4
	% ≥ 4	6
M7: percent relative abundance - Tanytarsini	% < 1	0
	1 ≤ % < 2	2
	2 ≤ % < 3	4
	% ≥ 3	6
M8: percent relative abundance - other Diptera and non-Insect invertebrates	% < 85	6
	85 ≤ % < 88	4
	88 ≤ % < 91	2
	% ≥ 91	0
M9A: percent relative abundance - tolerant taxa	% < 16	6
	16 ≤ % < 34	4
	34 ≤ % < 58	2
	% ≥ 58	0

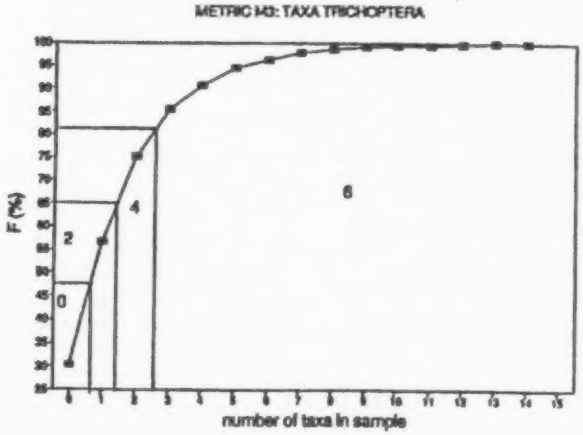
Table A1. (cont'd) - Metric value interval thresholds and corresponding scores obtained after calibration with the benthic data set from Ferraris (1984).

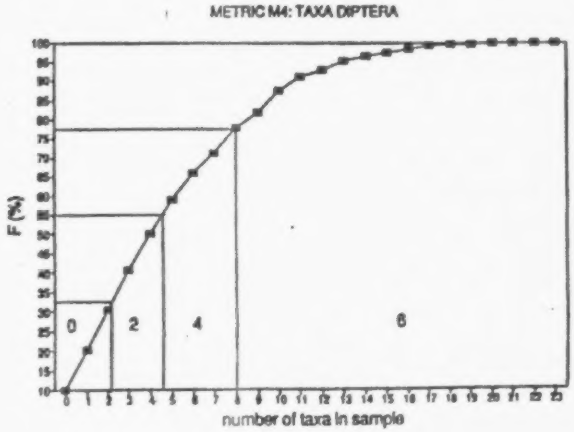
METRICS	THRESHOLD VALUES	SCORES
M9B: percent relative abundance - tolerant taxa	% < 16 16 ≤ % < 34 34 ≤ % < 58 % ≥ 58	6 4 2 0
M11: number of taxa - Gasteropoda	N = 0 N = 1 ou 2 N = 3 ou 4 N ≥ 5	0 2 4 6
M12: percent relative abundance - Gasteropoda	% < 3 3 ≤ % < 10 10 ≤ % < 27 % ≥ 27	0 2 4 6
M13: number of taxa - Oligochaeta	N = 0 ou 1 N = 2 N = 3 ou 4 N ≥ 5	6 4 2 0
M14: percent relative abundance - Oligochaeta	% < 3 3 ≤ % < 11 11 ≤ % < 32 % ≥ 32	6 4 2 0
M15: number of taxa - Hirudinea	N = 0 N = 1 N = 2 N ≥ 3	6 4 2 0
M16: percent relative abundance - Hirudinea	% < 1 1 ≤ % < 2 2 ≤ % < 3 % ≥ 3	6 4 2 0

Figure A1. Calibration graphs for metrics M1, M2 and M3.

LEGEND FOR FIGURES A1 TO A4	
F(%)	cumulative relative sample frequency (%)
0; 2; 4; 6	score values attributed to each class obtained by quadrisection of graph







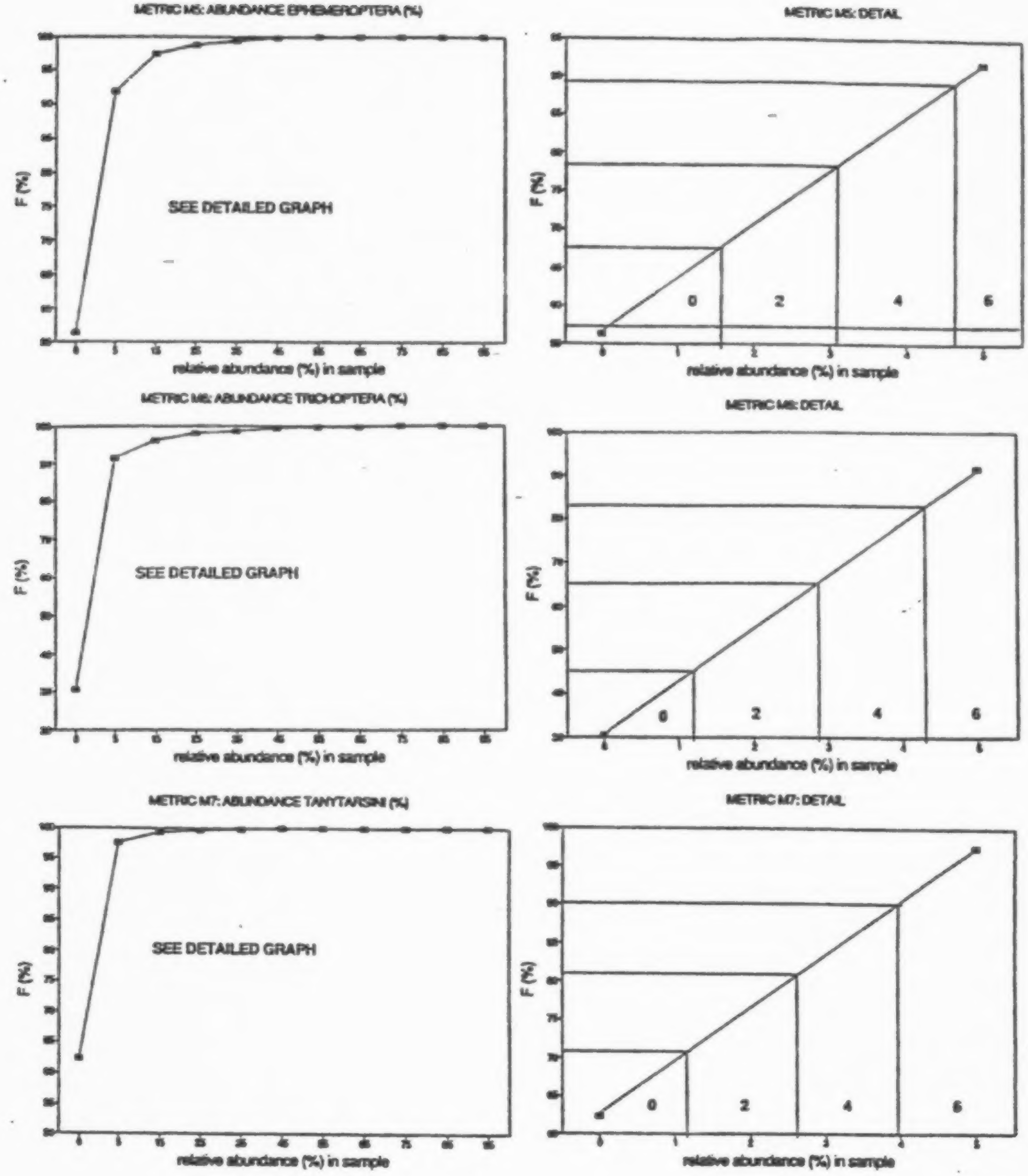


Figure A2. Calibration graphs for metrics M5, M6 and M7.

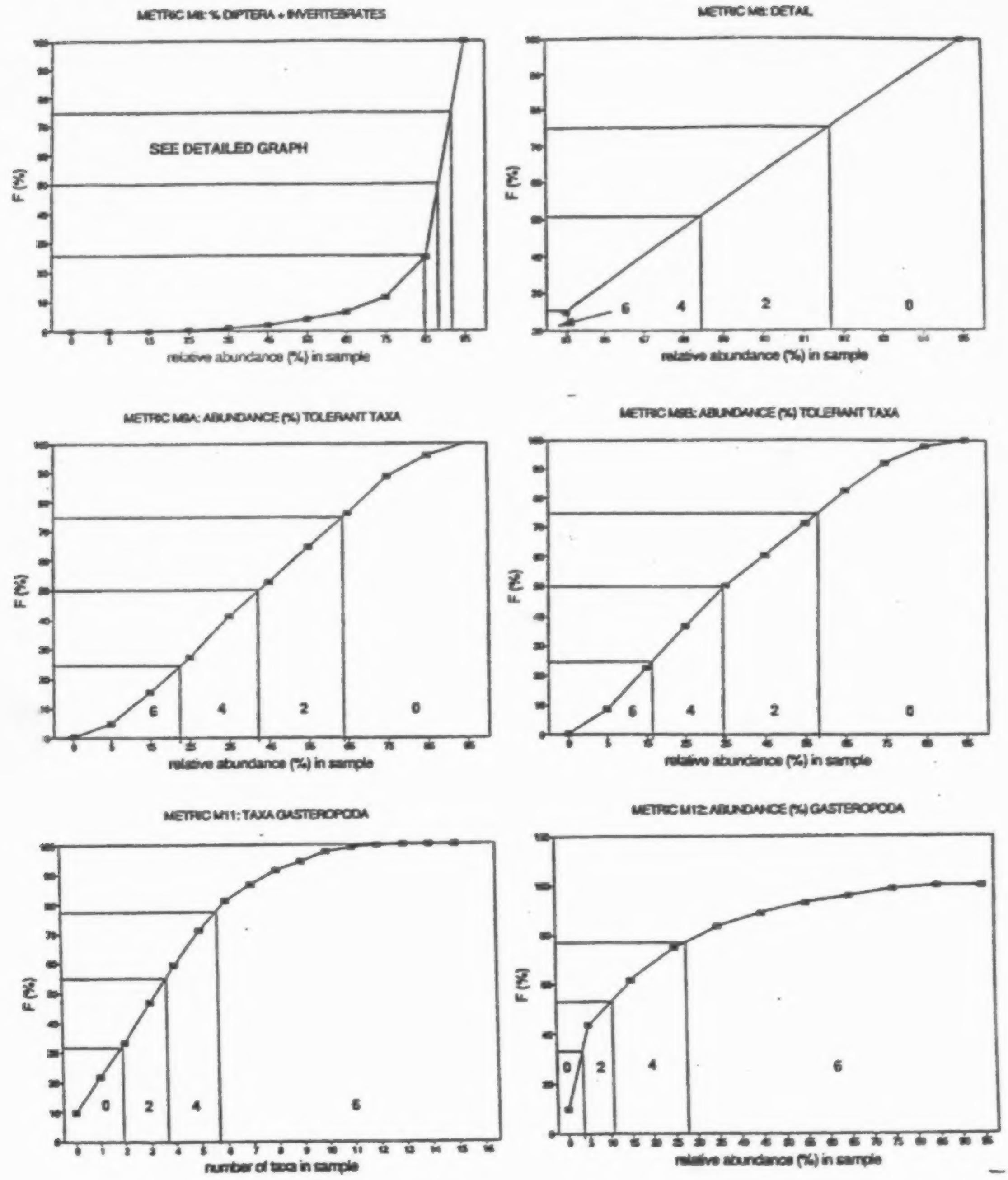
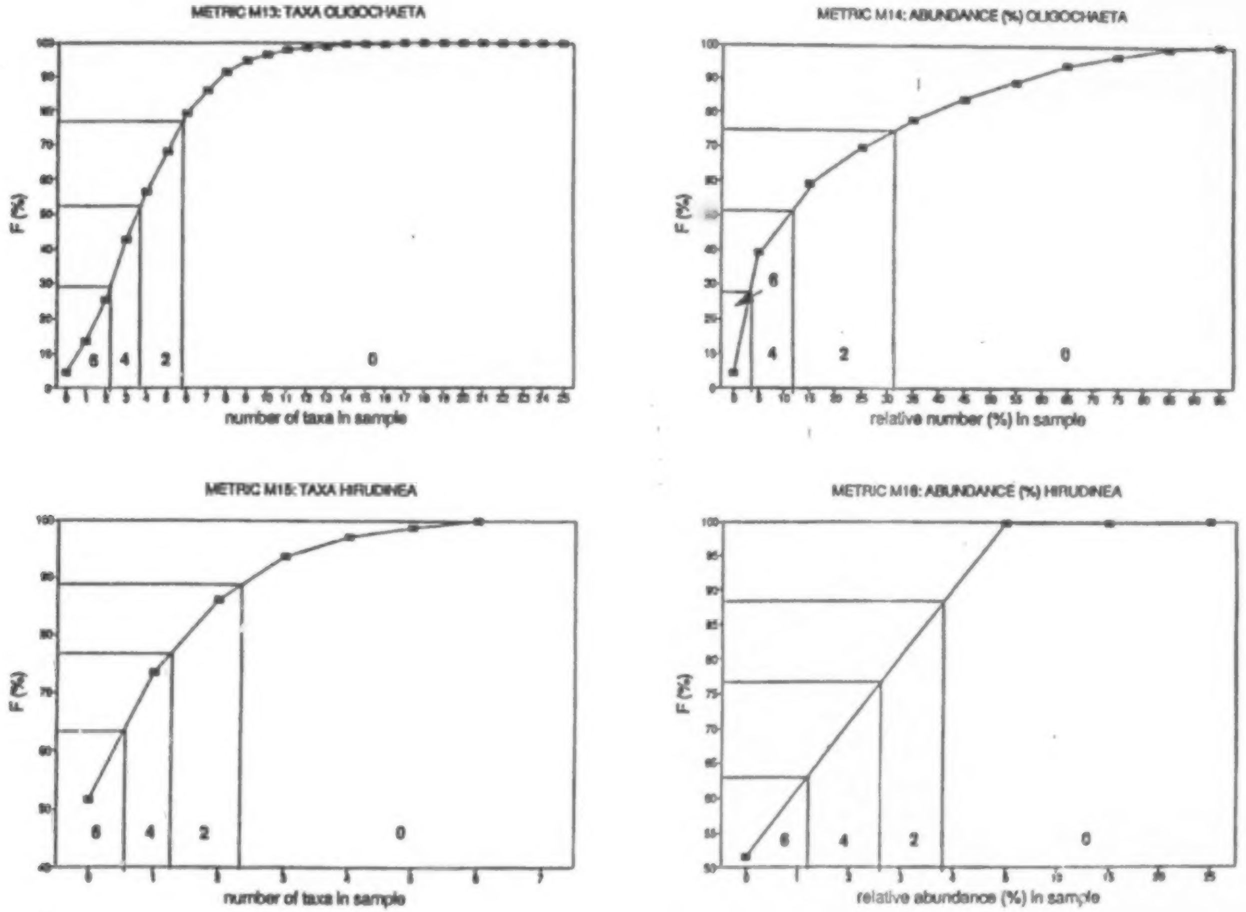


Figure A3. Calibration graphs for metrics M8, M9A, M9B, M11 and M12.

Figure A4. Calibration graphs for metrics M13, M14, M15 and M16.



PHYSIOLOGICAL EFFECT OF HIBERNIA CRUDE OIL DURING SMOLTIFICATION OF ATLANTIC SALMON *Salmo salar*.

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Healthy parr and smolts of Atlantic salmon *Salmo salar*, were monitored during a complete smolting cycle for basal mixed function oxidase (MFO) and ATPase activity. In addition, identical fish were experimentally exposed, during one complete smolting cycle (November to November), to different concentrations of Hibernia crude oil in water (0.0 - 6.3 ppm). In unstressed fish, MFO and ATPase activity varied considerably prior to, and rose to maximum levels, at smoltification; levelling off in late post-smolt fish. In oil-exposed fish, toxicity (24hr LC50 tests) of oil to the fish was not consistent, and differed with the smoltification stage of the fish. Pre-smolt fish were less sensitive (LC50 = 3.3 ppm) than post-smolt fish (LC50 = 2.2 - 2.7 ppm); fish in mid-smolt were least sensitive (LC50 = 5.3 ppm). In all instances, absolute sensitivity to oil in water was high, and fish displayed very narrow toxicity thresholds. Simultaneous enzymatic monitoring of the mixed function oxidase system in all stages showed obviously differing activity mainly during the smoltifying months (may-July), with total lower levels of cytochromes P450 and b5. BAPH activities during May through July appeared to be responding to oil levels in water, but dropped back to normal levels in late post-smolts. EROD activity did not display obvious oiling related changes.

EFFECTS OF INSTALLATION OF SECONDARY TREATMENT ON JACKFISH BAY WHITE SUCKER POPULATIONS EXPOSED TO BLEACHED KRAFT MILL EFFLUENT.

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Up until October of 1989, pulp mill effluent into Jackfish Bay, Lake Superior, received only primary treatment. White sucker *Catostomus commersoni* collected in 1988 and 1989 exhibited an increased condition factor and body fat, despite decreases in length at age and gonadal size. The fish showed increased hepatic MFO activity, decreased plasma sex steroid levels, and reductions in secondary sex characteristics and gonadal size. There were no differences in fecundity, fertilization rate, hatch, survival or development rate of larvae. Studies have continued during the first three years of secondary treatment. Although there has not been any biochemical indications of recovery, secondary treatment has been successful in mitigating more traditional impacts of effluent discharge. More than 90% of white sucker from Jackfish Bay show elevated levels of hepatic TCDD TEQs or EOX. Secondary treatment has been associated with limited improvement in liver size, condition factor and growth of younger age classes, although reproductive performance has been unaffected by secondary treatment. The persistence of biochemical changes after secondary treatment are consistent with the findings of other recent studies showing impacts of secondary-treated pulp mill effluents on other species in Jackfish Bay and on white sucker at other locations in Ontario.

EFFECTS OF THE EXXON VALDEZ OIL SPILL ON SURVIVAL OF PACIFIC HERRING EGGS AND VIABILITY OF THEIR LARVAE.

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INTRODUCTION

On March 24, 1989, the oil tanker Exxon Valdez spilled 250,000 barrels of Prudhoe Bay crude oil onto the surface of Prince William Sound, Alaska. During the first 60 hours the spill was confined to the center of the Sound. Then, a violent storm sent the slick moving rapidly southeast. By the fourth day (March 27), the slick was 60 km long and had begun passing through the Naked Island archipelago and by Knight Island. The slick began to exit the Sound by the end of the seventh day (March 30).

The spill coincided with the spawning period of Pacific herring (*Clupea pallasi*) in the Sound. Adult herring were first observed near their spawning grounds about one week after the spill (Alaska Department of Fish and Game 1991). Spawners were concentrated in four major areas. The Northeast area and the North areas were not touched by the spill, but the Naked Island archipelago and the northern tip of Montague Island were in the path of the spill.

Previous research has shown that growth and mortality of free-swimming herring larvae were not significantly different between oiled and non-oiled areas of the Sound (McGurk et al. 1993), suggesting that any effect of the oil spill on herring may have been restricted to the egg stage. We tested this hypothesis by measuring survival, hatching schedule and viability of herring eggs collected from oiled and non-oiled areas the Sound and incubated in laboratory aquaria.

MATERIALS AND METHODS

Herring eggs were taken from one control spawning beach in the North area, and five spawning beaches within the Naked Island archipelago and the northeastern tip of Montague Island. Between April 21-30, SCUBA divers from the Alaska Department of Fish and Game (ADFG) collected samples of herring eggs from 1-5 transects at each beach. They also recorded the presence or absence of oil, which was used to stratify egg samples into three oil classes: light, medium and heavy.

At depths of 1.5, 0.0, -1.5 and -4.6 m from mean lower low water divers laid a 0.1 m^2 frame on the substrate. Three separate handfuls of herring spawn were taken from within the frame and brought to the surface where they were immediately stored between layers of sea ice in insulated chests. The chests were flown to Seattle and then driven to the Vancouver Public Aquarium. Six chests containing over 180 samples from 20 transects were collected.

Herring eggs were incubated at 8.0-9.2°C and 26.8 ppt salinity in static aquaria with aeration and frequent replacement of water. Each bottle was examined every 1-2 days and all newly-hatched larvae were stored in 3.3% formalin. All eggs were counted with a dissecting microscope. Then, each bottle was filled with fresh seawater and placed back in its tub.

Larvae were sorted into normal and abnormal groups based on gross morphology, and the number of fish in each group was counted. A sub-sample of ten fish from the normal group was chosen for measurement of standard length, yolk length, and yolk height. Each larva was rinsed in freshwater, dried at 60°C for 24 h, and stored in a desiccator until it was weighed with an electrobalance. Yolk volume was calculated from the equation for an ellipsoid.

RESULTS

Initial egg numbers ranged from 49 to 1,074 with a mean of 289 (SD = 111, n = 180). Fractional survival of eggs ranged from 0.074 to 0.987 with a mean of 0.769 (SD = 0.173, n = 180). Since the mean hatching period was 6 d, eggs died at an average rate of $4\% \cdot d^{-1}$.

Analysis of variance (ANOVA) showed that egg survival varied with oil treatment, depth, and the interaction of treatment and depth. The oil effect was due to lower mean egg survival in the heavy-oil treatment than in the control treatment. The depth effect was due to lower mean egg survival at 1.5 m than at the other three depths, and higher survival at 0.0 m than at -4.6 m.

Based on the midpoints of the range of spawning dates estimated by ADFG, the average age of the eggs at collection ranged from 11 to 17 d with a mean of 14 d (SD = 2, n = 21). Eggs began to hatch within 1-2 d after they arrived in the laboratory. Duration of the hatching period ranged from 3 to 11 d, with a mean of 6 d (SD = 1, n = 180).

ANOVAs showed that mean age of hatch varied with oil treatment, depth, and the interaction of oil treatment and depth. The oil effect was due to earlier mean age at hatch for the heavy oil treatment compared to the control. The depth effect was due to greater mean age at hatch at -4.6 m compared to the other three depths.

Six larval abnormalities were identified: kinked or coiled spines; abnormalities of the yolk sac; missing or deformed jaw; short, stubby body; deformations of the head; and incomplete development of the caudal region of the body. Any one of these abnormalities would render a larvae effectively dead in a natural environment.

The fraction of viable larvae ranged from 0.346 to 1.000 with an arithmetic mean of 0.838 (SD = 0. 117). ANOVAs showed no significant variation of fractional viability with oil or depth. Mean larval length was 6.89 mm (SD = 0.98, n = 5,582). ANOVAs showed that mean length varied with oil and depth. The oil effect was due to longer mean length in the heavy-oil treatment than in the other three treatments. The depth effect was due to shorter mean length at -4.6 m than at the other three depths.

Geometric mean dry weight was 104 µg (n = 4,859), and geometric mean yolk volume was 0.0514 mm^3 (n = 5,545). Dry weight and yolk volume varied only with depth. This was due to greater mean weight and yolk volume at -1.5 m than at 0.0 m.

DISCUSSION

This study shows that the Exxon Valdez oil spill reduced egg survival and mean age at hatch, and caused an increase in mean length of viable larvae for heavily-oiled herring eggs. We conclude that the primary effects of exposure to oil were to kill herring eggs and to stimulate premature hatch of the surviving eggs.

Despite this finding, the population dynamics of both oiled and non-oiled herring eggs from Prince William Sound in 1989 resembled the dynamics of natural, uncontaminated herring eggs. The range of egg survivals is similar to ranges reported for medium and low densities of natural, uncontaminated herring spawn. Our average egg mortality rate of $4\% \cdot d^{-1}$ is in good agreement with most studies of natural herring spawn which show that mortality, due to causes other than predation is usually less than $10\% \cdot d^{-1}$. Our estimate of 83.8% mean larval viability falls within the range of 80-90% reported by other egg incubation studies.

One possible reason for the similarity in dynamics is that most eggs in the oil treatments may have been exposed to relatively low concentrations of hydrocarbons. Unfortunately, up to the present time we lacked accurate information on hydrocarbon concentrations. We are presently re-analyzing our results for 1989, 1990 and 1991 using a new hydrocarbon data set supplied by ADFG. We hope to stratify our samples into more tightly-defined classes of hydrocarbon concentrations and so demonstrate a stronger oil effect.

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McGurk, M. D., H. D. Warburton, V. Komori, and M. Galbraith. 1993. Growth and mortality of wild Pacific herring (*Clupea pallasi*) larvae in Prince William Sound, Alaska, after the (Exxon Valdez) oil spill. Can. J. Fish. Aquat. Sci. (In press)

ASSESSMENT OF BIOLOGICAL IMPACT FROM A WET LANDSCAPE OPTION FOR THE RECLAMATION OF FINE TAILS FROM OIL SANDS. M. MacKinnon, T. Van Meer, Syncrude Canada Ltd., Edmonton, AB, (403) 464-8490; A. Verbeek, University of Alberta, Edmonton, AB.

Syncrude Canada Ltd. operates an oil sands plant located in northeastern Alberta. Fluid wastes (water and fine tails) are a byproduct of the synthetic crude oil production. It is expected that over 600 Mm^3 of slowly consolidating fine tails (aqueous suspension of mineral tailings and unrecovered hydrocarbons) must eventually be reclaimed. Fresh tailings are acutely toxic to aquatic organisms. The main toxic components are released as a leachate from the oil sand during processing. They appear to biodegrade quickly under natural conditions. A wet landscape option is one of the reclamation methods being assessed as an environmentally acceptable method for handling the fine tails. In this approach, the fine tails would be transferred to below grade mined-out pits and capped with a layer of water. The depth of this water cap would be sufficient to allow the development of a viable and self-sustaining aquatic ecosystem. There are concerns about the release of toxic components from the fine tails and their impact on the capping waters. Both short term (acute) and long term (chronic) effects are being studied. Results from lab scale and long term field experiments using various bioassay methods will be described.

GROUNDWATER QUALITY MONITORING AT THE ALBERTA SPECIAL WASTE TREATMENT CENTRE, SWAN HILLS.

Lucien S. Lyness, P.Geol., Komex International Ltd., Calgary, AB, (403) 247-0200.

ABSTRACT

Groundwater quality is monitored by means of an array of observation wells. These wells are deployed in three monitoring intervals within a protective layer of clay till and in the underlying bedrock. Sampling accuracy is maximized by stainless steel well construction and dedicated sampling equipment.

Groundwater quality is monitored by periodically measuring the concentrations of selected organic and inorganic parameters. Organic parameters measured comprise gross indicators of organic loading, such as TOC and COD, as well as target compounds. Target compounds include PCBs and chlorobenzenes. Inorganic parameters measured include the main ions, total dissolved solids and trace metals.

At least two sampling visits take place per year. The resulting data are annually compiled and reviewed in detail with the support of statistical analyses. The annual reviews to date indicate that there has been no facility impact on groundwater quality.

The on-going monitoring program has provided useful information on the integrity and hydraulics of the key protective layer, especially as clay till is viewed as an important containment material throughout Canada. The effectiveness of individual chemical parameters has also been critically examined.

INTRODUCTION

The Alberta Special Waste Treatment Centre at Swan Hills is operated by Chem-Security (Alberta) Ltd. The facility is located in W1/2-6-67-8 W5M, about 13 km NE of Swan Hills townsite (Figure 1). Its role is as a central treatment operation which disposes of special wastes, particularly hazardous materials.

An extensive environmental monitoring network has been set up. Groundwater quality is included, thus a comprehensive program is used to monitor and establish if the facility is operating in an environmentally secure manner in respect to this important resource. Groundwater monitoring well (piezometer) locations are identified in Figure 2.

The main features of the facility are shown on Figure 2. The site can be roughly divided into two main areas. The process buildings are located on the northern part of the site, while an area of similar size to the south will eventually accommodate 40 engineered landfill cells. Both areas are fringed with groundwater monitoring wells. Wells 1, 2 and 4 through 10 are located on the periphery of the process area, and wells 1, 2, 3, 11, 12 and 13 surround the landfill area.

Figure 1. Location of Alberta Special Waste Treatment Centre at Swan Hills

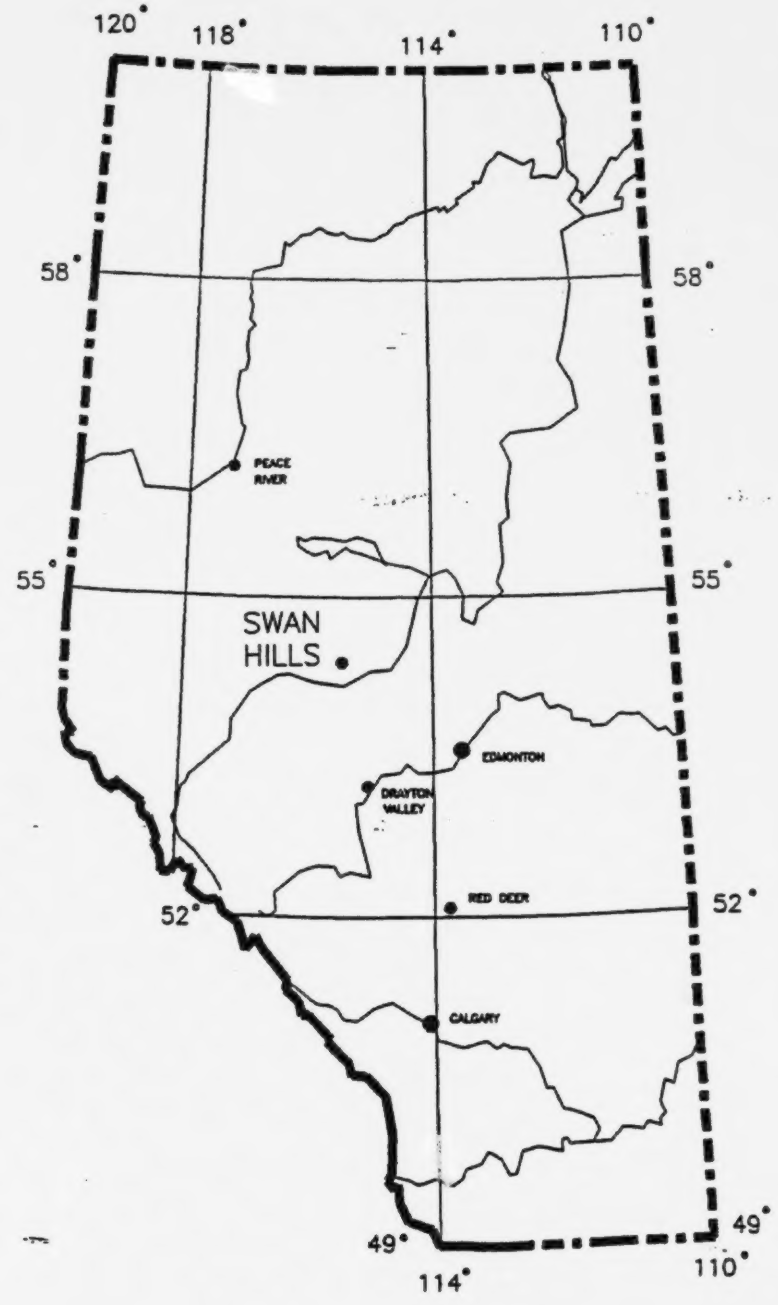
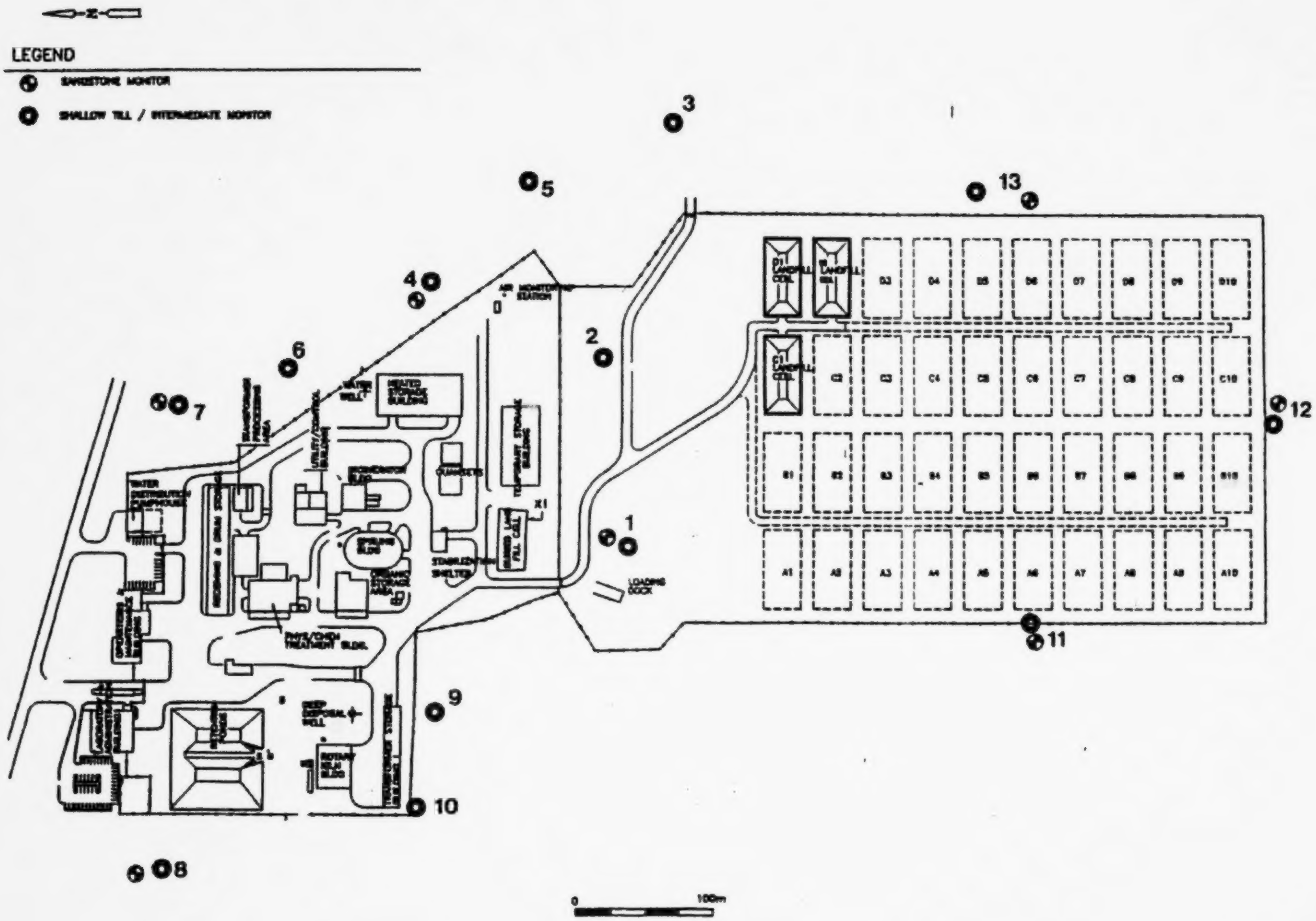

120°
118°
114°
110°
58°
58°
PEACE RIVER
55°
55°
SWAN HILLS
EDMONTON
DRAYTON VALLEY
52°
RED DEER
52°
CALGARY
49°
49°
114°
110°

Figure 2. Location of Groundwater Monitoring Sites


N
LEGEND
SANDSTONE MONITOR
SHALLOW TILL / INTERMEDIATE MONITOR
1
2
3
4
5
6
7
8
9
10
11
12
13
AIR MONITORING STATION
WATER WELL
HEATED STORAGE BUILDING
TRANSFORMER PROCESSING AREA
UTILITY/CONTROL BUILDING
INCINERATOR BLDG
QUONSETS
TEMPORARY STORAGE BUILDING
X1
WATER DISTRIBUTION PUMPHOUSE
STABILIZATION SHELTER
ORGANIC STORAGE AREA
PHYS/CHEM TREATMENT BLDG.
DEEP DISPOSAL WELL
ROTARY KILN BLDG
LOADING DOCK
D1 LANDFILL CELL
D2 LANDFILL CELL
D3
D4
D5
D6
D7
D8
D9
D10
C1 LANDFILL CELL
C2
C3
C4
C5
C6
C7
C8
C9
C10
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
0
100m

HYDROGEOLOGIC SETTING

Bedrock

The site vicinity is underlain by strata associated with the basal succession of the Paleocene Paskapoo Formation. This succession includes the basal Scollard Member (Green, 1972). The Paskapoo Formation consists of thick-bedded calcareous, cherty sandstone; siltstone and mudstone; and minor conglomerate, thin limestone, coal and tuff beds. The Scollard Member consists of feldspathic sandstone, bentonitic mudstone and thick coal beds. It is difficult to estimate the remaining thickness of Paskapoo Formation strata under the facility based on available published geologic information. However, judging by the hydrogeologic cross-sections and maps provided by Tokarsky (1977), it is probably somewhat less than 100 m.

The Paskapoo Formation is underlain by the Wapiti Formation. This unit comprises feldspathic, clayey sandstone; bentonitic mudstone and bentonite; and a few coal beds. The structure is uncomplicated, the strata having a gentle dip of about 0.2° to the southwest. As a result of this dip, in combination with a decline in topographic elevation, the subcrop of the Wapiti Formation occurs at a relatively short distance to the north, east and south of the site.

Quaternary Deposits

The most prevalent surficial (Quaternary) deposit throughout the Swan Hills region is unstratified glacial drift (mostly till) of the Pleistocene epoch (St. Onge, 1973). The till is represented by hummocky and ground moraines. These, however, are fairly thin, as drift thicknesses in the area are reported as being generally less than 15 m (Carlson and Green, 1977).

Aquifers

As regards potential potable water supplies, the most important unit is the Paskapoo Formation, which is the subcropping bedrock unit throughout the westerly part of the site region. It contains occasional water-bearing layers or horizons. Tokarsky (1977) rates its yield potential as being in the range of 0.4 to 2.0 L/sec. This is a good yield potential by domestic (household) standards. In general, the underlying Wapiti Formation has a comparatively poor yield potential of less than 0.1 L/sec.

Therefore, the Paskapoo Formation represents the major potential source of potable bedrock groundwater supply regionally. However, this resource is little used due to the relatively low population density in the area. The Quaternary deposits, being rather thin and mostly composed of till, offer little water supply potential in the region.

Water Quality

Tokarsky (1977) presents hydrochemical maps for water well depth intervals of 0 - 15 m, 15 - 60 m and >60 m for the general region. However, control points for the Swan Hills area are very sparse, hence these regional interpretations must be treated with caution local to the facility. The map for shallow bedrock (i.e. 15 - 60 m below ground level) suggests that the water is of relatively good quality since a mineralization of less than 500 mg/L TDS and a bicarbonate type are indicated. Less data appear to be available to support interpretations for the other two intervals in the vicinity of the facility.

The two key geologic units underlying the facility are hence a protective layer of clay till which overlies a water-bearing basal sequence of Paskapoo Formation strata. Three geologic intervals are monitored at the facility. These are:

Stratigraphy	Dominant Local Lithology	Hydrogeologic Unit	Monitoring Interval Below Ground Level	
Pleistocene:	Clay till	Aquitard	1.	3-4 m (shallow)
Hummocky Moraine (Wisconsin Stage)	Clay till	Aquitard	2.	6-9 m (intermediate)
Paleocene:	Sandstone	Aquifer	3.	58-67 m (sandstone)

The comparatively low permeability containment layer is thus monitored at two levels. The underlying bedrock unit is monitored at the upper part of the saturated zone.

SPECIAL FEATURES OF THE MONITORING WELLS

The monitoring wells, and attendant sampling equipment, are constructed for optimum sample integrity. The well screens and tubing are all stainless steel and have an industry standard diameter of 50 mm. These are equipped with dedicated sampling devices, thus avoiding the possibility of sample cross-contamination. The leading sampling device is the bladder pump system. The bladder pumps are an assembly of teflon and stainless steel components.

Because of the relatively recent sensitivity of environmental issues throughout North America, most aspects of environmental hydrogeology have been the subject of detailed research. The Swan Hills facility operators have utilized state-of-the-art groundwater quality monitoring techniques from project inception. For example, stainless steel and teflon have been shown to be superior materials for specialized work. The bladder pump system has also been shown by detailed research to provide the most accurate sampling results of all common available sampling devices. It is an elegant sampling technique whereby the sample comes to surface under positive pressure, thus tending to retain potential volatile constituents in solution. As well, the carrier gas is never in contact with the sample. To ensure a consistent level of high accuracy and reproducibility, the sampling process adheres to formal sampling protocols and quality control measures.

In short, the Swan Hills groundwater monitoring system provides rare access to data of the highest quality on groundwater chemistry and aquifer/aquitard hydraulics in Alberta.

HYDROCHEMICAL DATABASE

Prior to the official opening on September 11, 1987, baseline data were collected for the period of February 1985 to September 1987. Since then, data have been collected either four times or twice per year. Over the years, an impressive array of hydrochemical data has thus been compiled.

A comprehensive schedule of chemical analyses includes both organic and inorganic parameters. Organic parameters measured include gross indicators of organic loading, such as total organic carbon (TOC) and chemical oxygen demand (COD). As regards target compounds selected for analysis, focus is given to those materials which are commonly processed at the facility. These include organic compounds such as polychlorinated biphenyls (PCBs) and a suite of chlorobenzenes. Inorganic parameters measured include the main ions, total dissolved solids and secondary and trace metals.

IMPACT OF FACILITY OPERATIONS

At the end of each year, the newly acquired data set is evaluated in the context of the database generated from the baseline and previous annual information. The data are reviewed both piecemeal and with the aid of statistical analyses. To date, the concentrations of the various indicator parameters largely remain within the limits of statistical control, while no confirmed positive readings of target compounds have been recorded. It has therefore been concluded by project reviewers that in respect to the parameters measured, the facility has had no observable impact on groundwater quality as measured at the monitoring wells.

AQUIFER/AQUITARD HYDRAULICS

Typically, groundwater movement in shallow till is closely controlled by local topographic relief. As expected, therefore, the groundwater flow direction (Figure 3) is consistent with the decline in topographic elevation from west to east towards the Coutts River (Figure 4). A previous review of the historic data indicated that water levels are slightly lower over the winter months (Integrated Environments, 1992). In general, water levels are comparatively high in the late spring/summer months, and this would coincide with spring recharge. Groundwater movement in the intermediate interval is similarly controlled by topographic relief, the groundwater flow direction also being eastward towards the Coutts River.

Throughout the years, it has been found that water levels in the shallow and intermediate piezometers have generally remained at much the same overall elevation (eg. Figure 5). In contrast, the sandstone piezometer readings indicate that a gentle decline has taken place since 1985 (eg. Figure 5). This expected local decline would be related to groundwater abstraction from Paskapoo Formation sandstone for use by the facility. No comparable flow pattern is thus discernable for this unit.

In some instances the hydrographs for the shallow and intermediate zones show close sympathetic responses (eg. Figure 6). This feature is most evident where the respective water levels are at much the same elevation. Where the groundwater surface is at a lower

Figure 3. Distribution of Groundwater Surface Elevations Shallow Piezometers

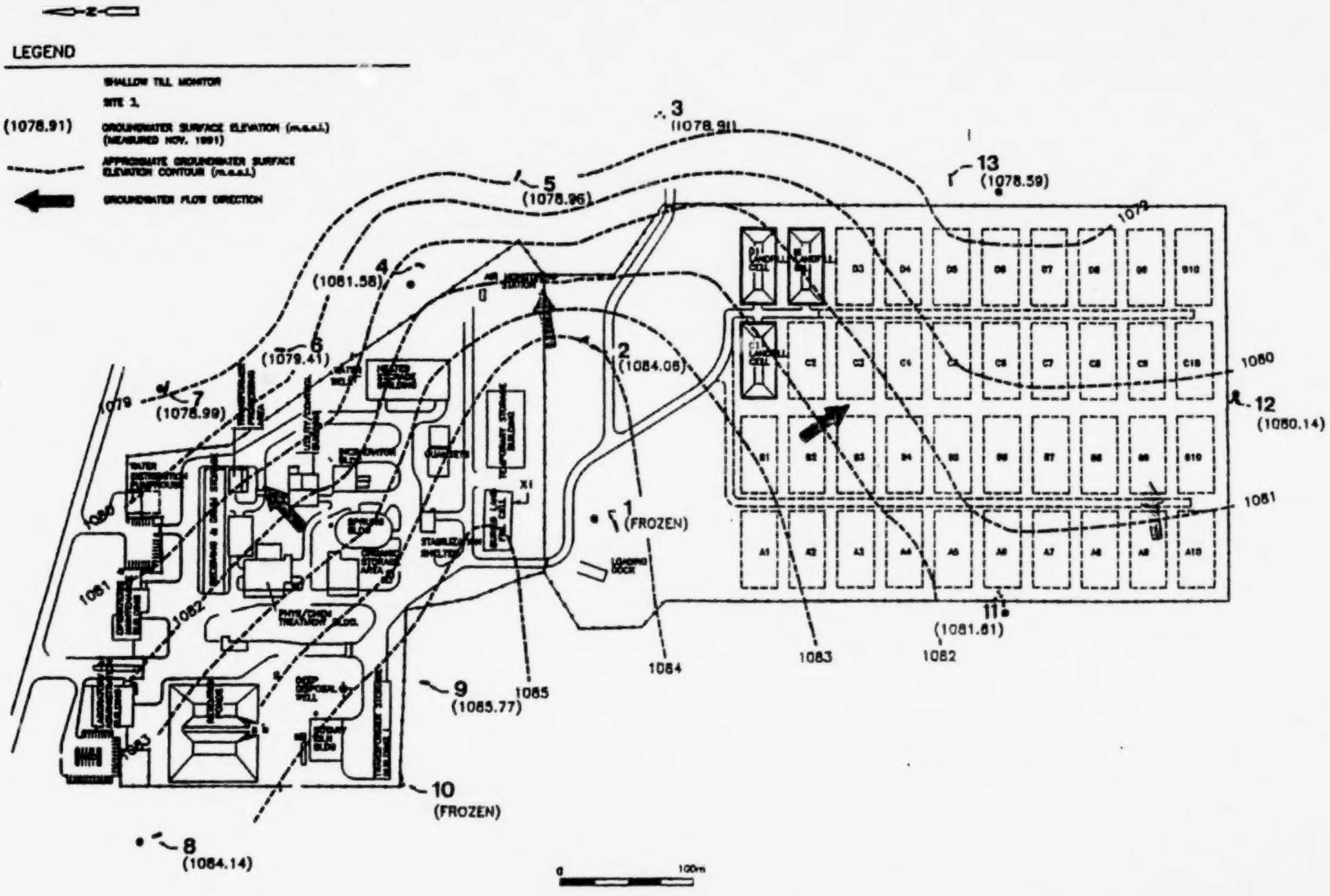
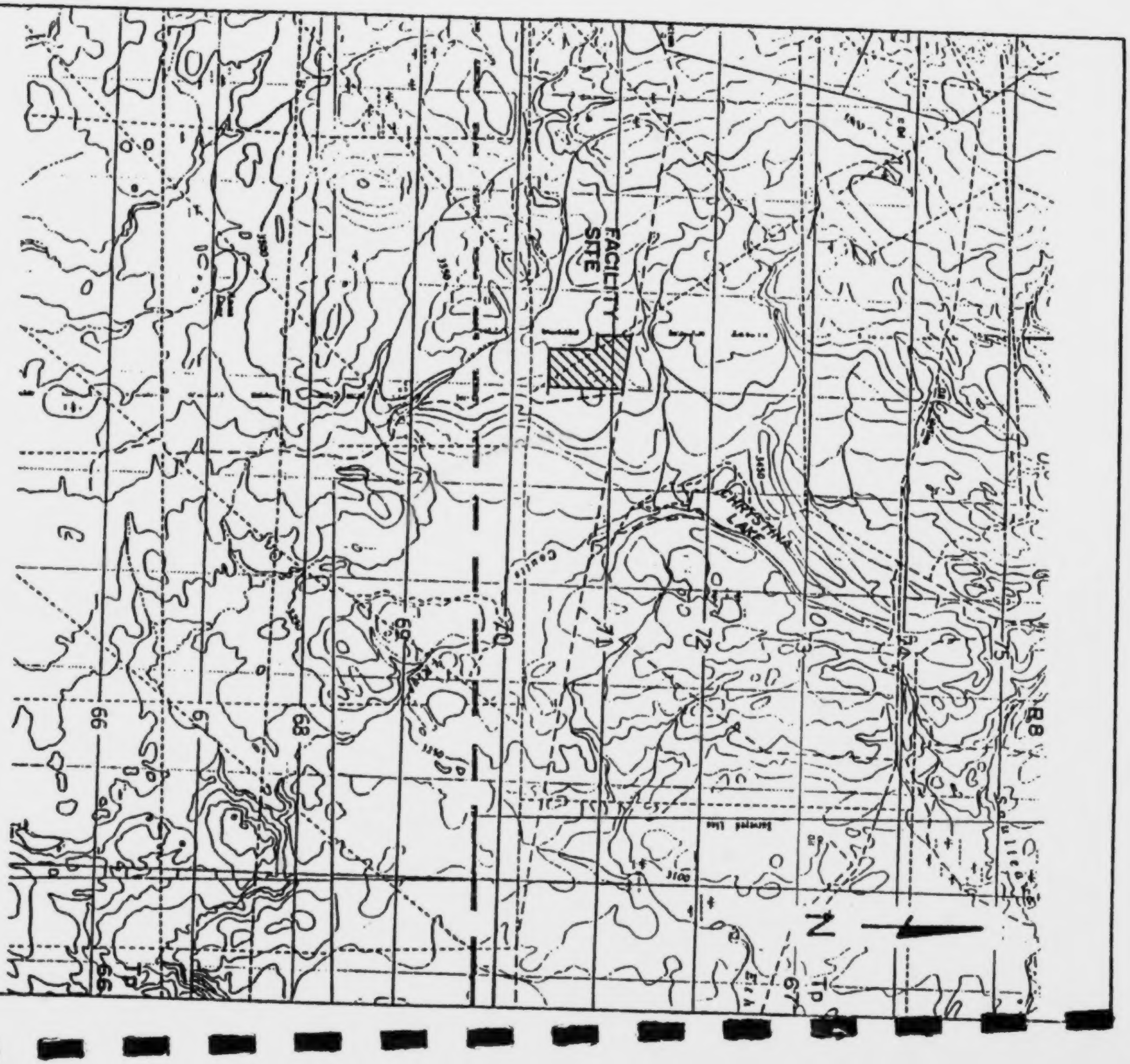

N
LEGEND
SHALLOW TILL MONITOR
SITE 1.
(1078.91) GROUNDWATER SURFACE ELEVATION (m.a.s.l.) (MEASURED NOV. 1991)
APPROXIMATE GROUNDWATER SURFACE ELEVATION CONTOUR (m.a.s.l.)
GROUNDWATER FLOW DIRECTION
3 (1078.91)
5 (1078.96)
13 (1078.59)
4 (1081.58)
6 (1079.41)
7 (1078.99)
2 (1084.06)
12 (1080.14)
1 (FROZEN)
11 (1081.81)
9 (1085.77)
10 (FROZEN)
8 (1084.14)
1079
1080
1081
1082
1083
1084
1085
D3 D4 D5 D6 D7 D8 D9 D10
C2 C3 C4 C5 C6 C7 C8 C9 C10
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10
LANDFILL CELL
WATER INLET
LOADING DOCK
STABILIZATION SHELTER
PHYS/CHEM TREATMENT BLDG.
DEEP DISPOSAL WELL
X1
0
100m

Figure 4.


FACILITY SITE
CHRYSTINA LAKE
N
66
67
68
69
70
71
72
73
74
75
TP 66
TP 67
R8

LOCAL PHYSIOGRAPHY, (1:50 000)

elevation in the intermediate zone than in the shallow zone (eg. Figure 5), a more pronounced fluctuation appears to take place in the intermediate zone. Seasonal fluctuations are not so apparent in the sandstone (eg. Figure 5), a gentle decline being the predominant trend in this unit.

Groundwater levels in the intermediate zone are either at the same elevation as those of the shallow zone (eg. Figure 6) or lower (eg. Figure 5). A lower groundwater surface in the intermediate zone indicates a downward flow component or potential recharge configuration in or through the till. Where the two groundwater surfaces coincide, groundwater flow can only take place in the horizontal plane.

The groundwater surface in the sandstone is in the order of 50 m lower than in the till (eg. Figure 5). Thus the till, as an aquitard with a downward flow component, has potential to convey recharge to the bedrock. However, the present vigilant monitoring of the water quality in the bedrock of course routinely provides a means of detecting any facility-related constituents that may accompany the recharge.

MAIN ION CHARACTERIZATION

Apart from usual comparatively small seasonal fluctuations, natural groundwater chemistry is very stable spatially and temporally within a given aquifer. It typically shows only gradual changes or trends along the flow path, with increased residence time. The major ions (Ca^{2+}, Mg^{2+}, Na^{+}, HCO_3^-, Cl^-, SO_4^{2-}) normally make up 99% of the dissolved species in natural groundwater. Commonly, delineation of recharge areas and mixing zones can be done by hydrochemical facies mapping based on the fingerprint provided by the main ions. Similarly, main ion chemistry can be effectively used to determine if groundwater is being impacted by anthropogenic sources, and to what degree. Even small impacts on groundwater quality can be identified through proper scrutiny of main ion chemistry.

The hydrochemical nature of the groundwater samples from the Swan Hills facility has been characterized on an expanded Durov diagram (Figure 7). This type of diagram provides a simple, concise graphical presentation of water analyses, and allows each sample to be classified as a distinct hydrochemical type. A main advantage of this diagram is that water quality data points plot in well-defined fields that exist in nature. These data points are plotted according to the proportions of individual major cations (Ca, Na, K, Mg) and anions (HCO_3, SO_4, Cl) in % millequivalents per litre (meq/L).

Recently recharged water in many aquifers is commonly a calcium-bicarbonate hydrochemical type. This plots in the top lefthand square of the Durov diagram. Natural softening of the water via ion exchange (Na for Ca) to a sodium-bicarbonate hydrochemical type generally occurs with increasing residence time in the aquifer (top righthand square of the Durov diagram). Oldest waters are commonly dominated by a sodium-chloride type, and generally plot in the lower righthand square of the diagram. These waters can represent stagnant, connate or pseudo-connate types.

Another well-known hydrochemical evolution sequence relates to the change in dominant anion species with residence time in aquifer (Freeze and Cherry, 1979). This evolution is represented by the following sequence: (Youngest) HCO_3 - SO_4 - Cl (Oldest)

Figure 5. Hydrographs of Piezometers 1-4P, 1-9P and 1-65S, (Site 1)

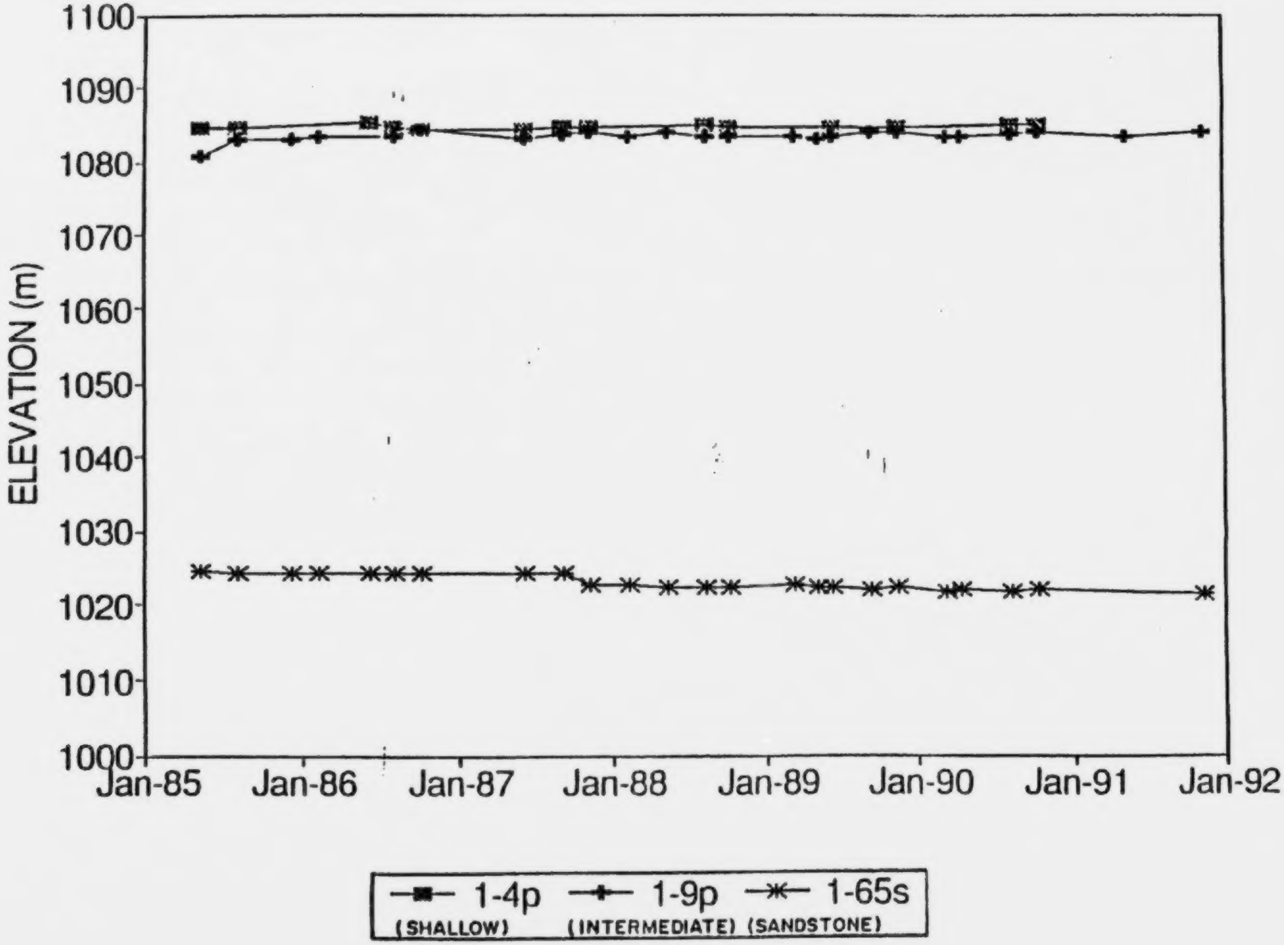
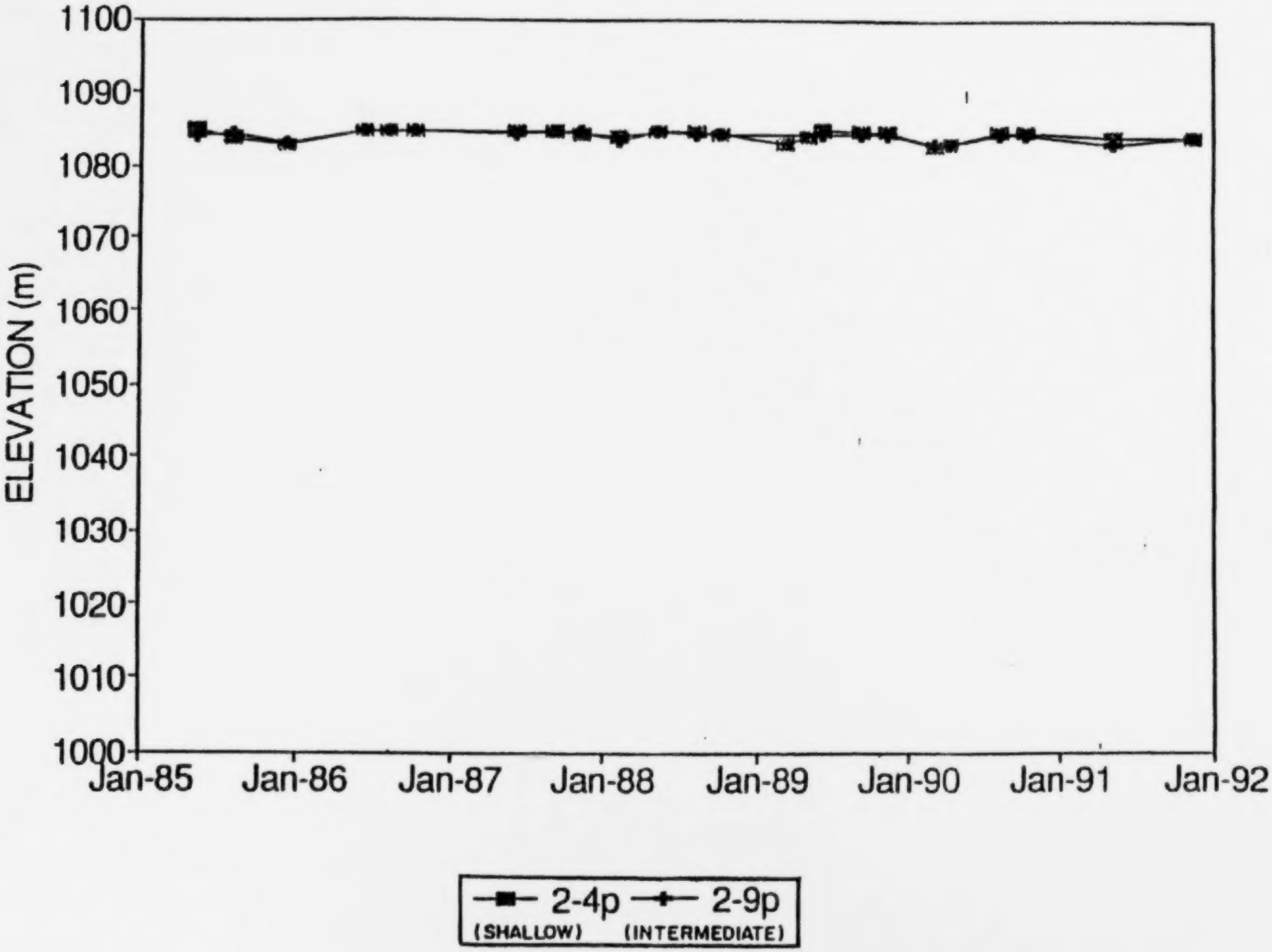


Figure 6. Hydrographs of Piezometers 2-4P and 2-9P, (Site 2)



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WATER QUALITY AND SEDIMENT CHEMISTRY MONITORING AT THE ALBERTA SPECIAL WASTE TREATMENT FACILITY, SWAN HILLS, ALBERTA.

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ABSTRACT

The 1991 surface water quality and sediment chemistry monitoring programs at the Alberta Special Waste Treatment Facility are presented, and their role as part of the overall environmental monitoring program will be discussed.

Currently, 10 sites around the facility are monitored as part of a program commenced in 1985 (prior to facility start-up in 1987).

Water quality at most monitoring sites remains in general very good, though the facility appears to have had a small impact on the quality of the closest sites, two drainage ditches directly adjoining the facility. At the remaining sites, some parameters were found to be elevated above background levels. There is, however, considerable scatter in the data, and levels are generally quite low.

1991 was the first year in which the sediment samples were tested for specific chlorinated hydrocarbons, including 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, and PCBs. Very low levels (near the detection limit) of these target compounds were detected at many sites. The possible sources of these compounds will be discussed.

DESCRIPTION OF THE FACILITY

The Alberta Special Waste Treatment Centre is a fully integrated hazardous waste treatment facility located near the geographical centre of the province. The plant provides comprehensive treatment for any hazardous waste produced in the province of Alberta.

Siting of the facility began in the early 1980's. Construction commenced in 1985, with facility start-up in the fall of 1987.

The site lies approximately 20 km northeast of the Town of Swan Hills, in an area of dense glacial deposits overlain by muskeg and boreal forest. The area around the plant is widely used for forestry and oil and gas exploration and processing.

The plant receives waste of many types: organic and inorganic; solids, liquids and sludges. The waste treatment scheme selected depends on the physical and chemical characteristics of the waste. Organic liquids and organics-contaminated aqueous and solid wastes are incinerated. Aqueous inorganic waste is treated in the Physical/Chemical treatment facility, while solid inorganic waste is treated in the Fixation/Stabilization facility. Known mercury-contaminated wastes are not currently treated, but are being stored on-site.

Stabilized Solids and incinerator ash from processing are deposited in landfills. Treated aqueous waste and blowdown water from the incinerator flue gas scrubbing system are deep-well injected.

The only engineered source of emissions from the plant is the incinerator stacks. The stacks are equipped with scrubbers, and are continuously monitored for SO_2, CO, and total hydrocarbons. The primary sources of emissions has become fugitive emissions: generally materials readily transportable by air (typically volatile and semi-volatile organic compounds and inorganic dusts). The sources of these emissions range from wastes in storage (eg. leaking drum bungs), waste unloading (both bulk and drum decanting) and the introduction of waste to the processing units (eg. kiln feeding and stabilization).

The site is divided in two areas: the "plant", where processing and storage of untreated waste occurs, and the landfill area, where processed solid wastes are stored. The plant area is designed such that all surface water is directed to a stormwater retention pond, and used by the plant. All water not used by the plant is deep-well injected. Water from the landfill cell area is not contained within the plant; this water joins the natural drainage toward the southeast.

The main transportation mechanism for emissions is wind, which blow primarily from the WNW across the site. However, on warm days a diurnal variation occurs caused by anabatic heating resulting in winds from the SE. The stack emissions will tend to be dispersed further afield than the fugitive emissions because of the temperatures (30-80°C versus ambient) and elevation (15 m) of release. Water may be an important secondary transport mechanism, as the drainage in the plantsite area is generally to the south and southeast. Thus, contaminants may be carried by water to areas little affected by wind.

OVERVIEW OF THE ENVIRONMENTAL MONITORING PROGRAM

A comprehensive three-tier monitoring program has been established at the site, and includes the following components:

1. waste assessment, Treatment Centre personnel sampling, health surveillance, and Industrial Hygiene monitoring;
2. plant boundary air quality monitoring;
3. biological and chemical monitoring in the vicinity of the plant.

The first tier deals primarily with worker health and safety and consequently beyond the scope of this paper. The other items pertain to an integrated environmental monitoring program (Chem-Security, 1991) outlined below.

An ambient air quality and meteorological monitoring program has been on-going since the construction phase. Construction phase data were used the delineate relative impact zones based on stack dispersion modelling. In addition, these data form the "background" data against which current data are compared. A number of parameters are monitored continuously at various locations around the site: SO_2, H_2S, NO & NOx, CO and total hydrocarbons). In addition, samples for particulates and PCBs are collected over a 24-hour composite period every sixty days.

The wide variety of waste processes at the plant makes environmental monitoring problematic. Consequently, off-site monitoring focuses on parameters which are particularly stable in the environment. For this reason, in addition to the usual indicator compounds, heavy metals and PCBs are the parameters of primary concern.

The biological and chemical monitoring in the vicinity of the plant consists of the following components:

Soil: sampling of the moss, litter, humic and mineral soil (to 1 m) layers.
Vegetation: sampling of Labrador tea leaves, and lichen coverage.
Wildlife: sampling of the Gapper's red-back vole. It was through the monitoring of this animal that the importance of fugitive emissions was first realized.
Water quality and sediment: sampling of water quality and sediment from 9 stream and 1 lake site.
Benthic invertebrates and fish: fish tissue (trout and sucker chemistry) from 1 lake, and benthic community structure.

WATER QUALITY

INTRODUCTION

The surface water sampling network was established in 1985. Prior to facility start-up in the fall of 1987, baseline data were collected. The monitoring program continues to collect operational monitoring data, though the frequency of monitoring has been reduced to the current twice per year monitoring.

The objective of the program is to monitor any impacts the Treatment Centre might have on nearby surface waters. Since all drainage from the active part[1] of the site is diverted to the stormwater pond, impacts on surrounding waters may be expected primarily from three sources: i) incinerator stack emissions; ii) runoff from the landfill cell area; and iii) airborne fugitive emissions.

The ten monitoring sites are all located within 2.5 km of the Treatment Centre (Figure 1). The study streams are generally low order, low gradient tributaries of the Coutts River. They drain the muskeg and related wetlands that surround the facility. There is one lake site north-east of the facility. Chrystina Lake is a popular recreational fishing site 1.5 km north east of the facility. It is a narrow, spring fed lake with no significant tributaries. The water level is controlled by a weir and has been raised recently, flooding soils. There is currently a notice in place warning anglers about limiting consumption of fish from the lake due to mercury concentrations.

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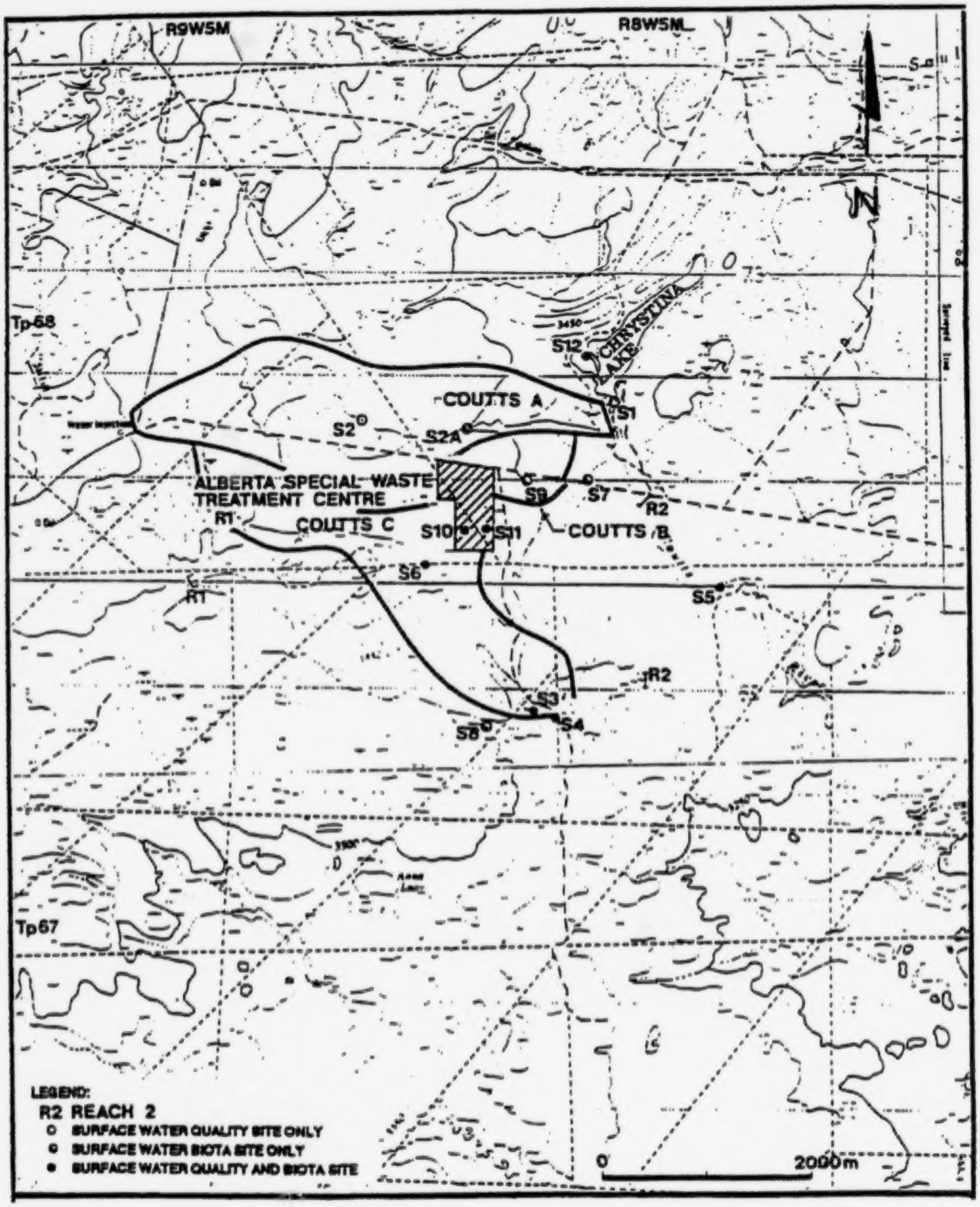
[1] the plant site consists of an active area where the incinerator, loading/unloading facilities, etc. are located, and a landfill area, where processed materials are interred.

Figure 1. Surface Water Monitoring Location Plan


R9W5M
R8W5M
N
Tp 68
CHRYSTINA LAKE
S12
S1
COUTTS A
S2
S2A
ALBERTA SPECIAL WASTE TREATMENT CENTRE
R1
COUTTS C
S10
S11
S9
S7
R2
COUTTS B
S6
S5
R1
R2
S3
S4
S8
Tp67
LEGEND:
R2 REACH 2
SURFACE WATER QUALITY SITE ONLY
SURFACE WATER BIOTA SITE ONLY
SURFACE WATER QUALITY AND BIOTA SITE
0
2000m

Two sites, S10 and S11, are drainage ditches which border the landfill area of the Treatment Centre on the west and east, respectively.

Two sites, S2 and S6 (northwest and southwest of the facility) are upstream of the site, and were initially intended as background sites. Recently, however, wind data at the site were re-analyzed, showing that while the predominant wind direction is toward the southeast, there is a diurnal variation in direction toward the northwest. Thus, S2 may no longer be considered a background site.

METHODS

Water samples are collected from each of the 10 locations. Samples are collected slightly below the water surface, refrigerated, and delivered to the receiving laboratory, Chemex Labs Ltd., within a 7-day period.

The current analytical program includes analyses for the parameters shown in Table 1. Spring sampling (May) includes only indicator parameters (pH, conductivity, total organic carbon, and total dissolved solids), while in Fall (September) the full suite of parameters is employed.

In previous years, priority pollutant scans of surface waters were performed. No halogenated or nitrogen/phosphorus-based organic compounds have been identified in surface waters since the start of monitoring. Phthalate or adipic acid esters have been identified, but are believed to be laboratory artifacts. Priority pollutant scans are not part of the current protocol.

RESULTS AND DISCUSSION

In general, sites beyond the immediate vicinity of the plant show no discernible trend in water quality since the start of monitoring. Many parameters (particularly metals) were found at or near detection limits, and well below the Guidelines for Freshwater Aquatic Life (CCREM, 1991).

There is evidence of degraded water quality at three sites. Figures 2 through 5 show the plots of sulphate, chloride, total organic carbon, and chemical oxygen demand for each site for all time periods. Site S10, a drainage ditch bordering the landfill cell area on the west, clearly showed evidence of hydrocarbon contamination. Subsequent investigations identified the probable source of contamination as a fire training area situated in the landfill area. Site S11, the eastern drainage ditch through which much of the landfill cell area drains, also exhibited elevated levels of a number of parameters, including chloride and sulphate. The highest recorded sulphate levels in surface waters were located at S10 and S11 (less than 80 mg/L), but were well within acceptable limits. Interestingly, site S9 showed quite low sulphate levels, despite having been included among the monitoring sites due to its location within the predicted sulphate deposition plume. Chloride levels at this location were very slightly elevated, but very low.

Figure 2. Sulphate Surface Water

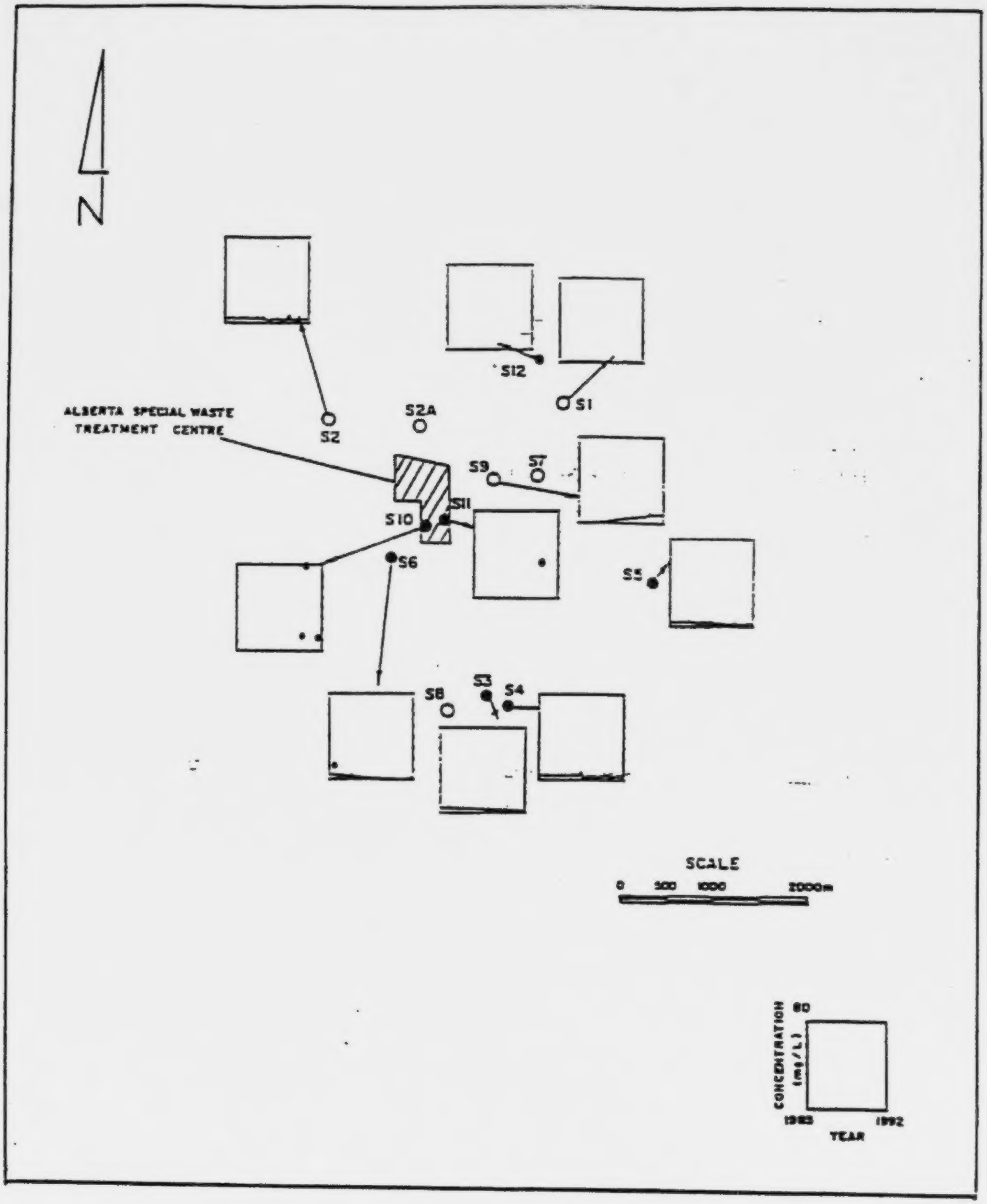

N
ALBERTA SPECIAL WASTE TREATMENT CENTRE
S1
S2
S2A
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
SCALE
0
500
1000
2000m
CONCENTRATION (mg/L)
80
1983
1992
YEAR

Figure 3. **Chloride Surface Water**

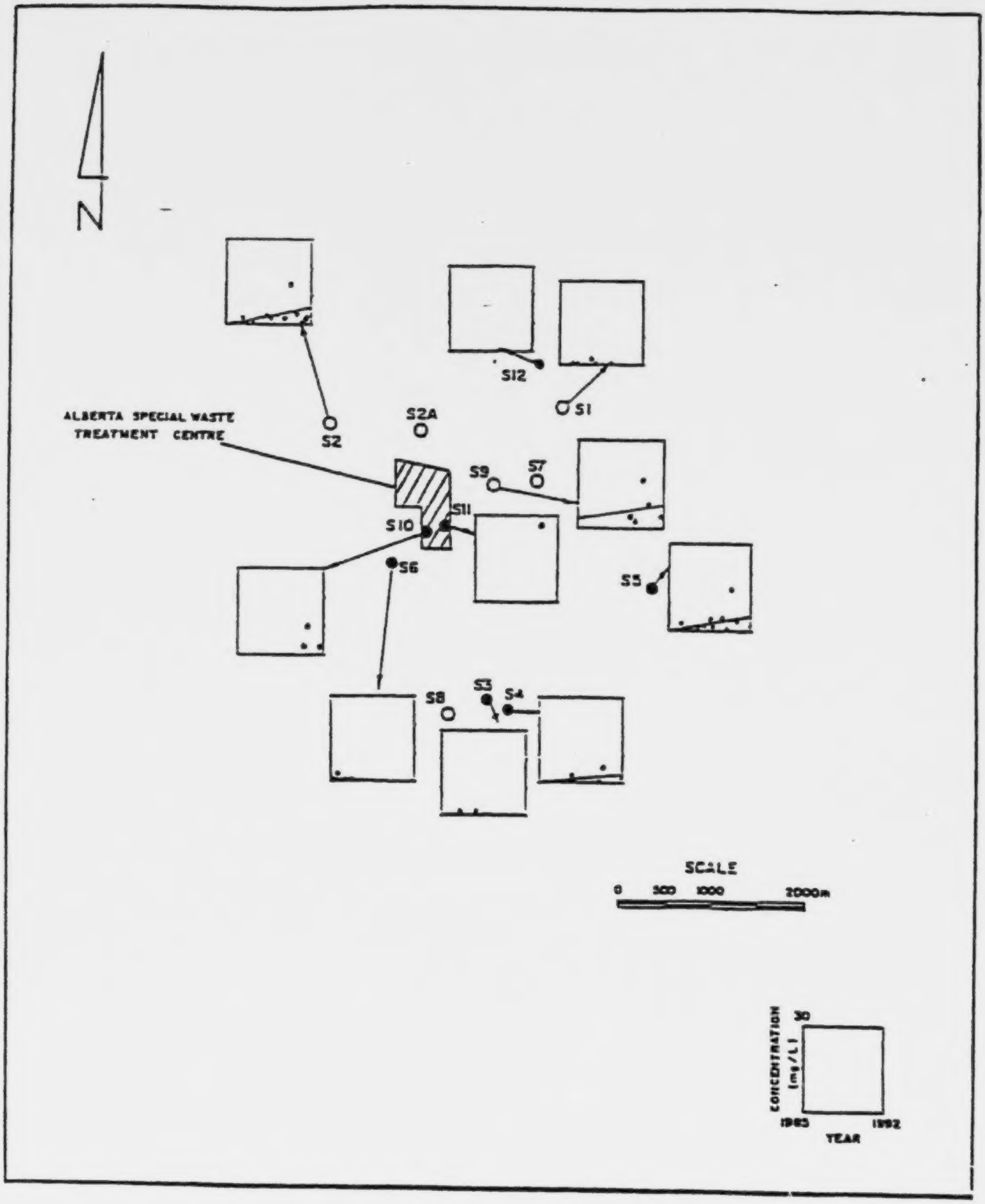
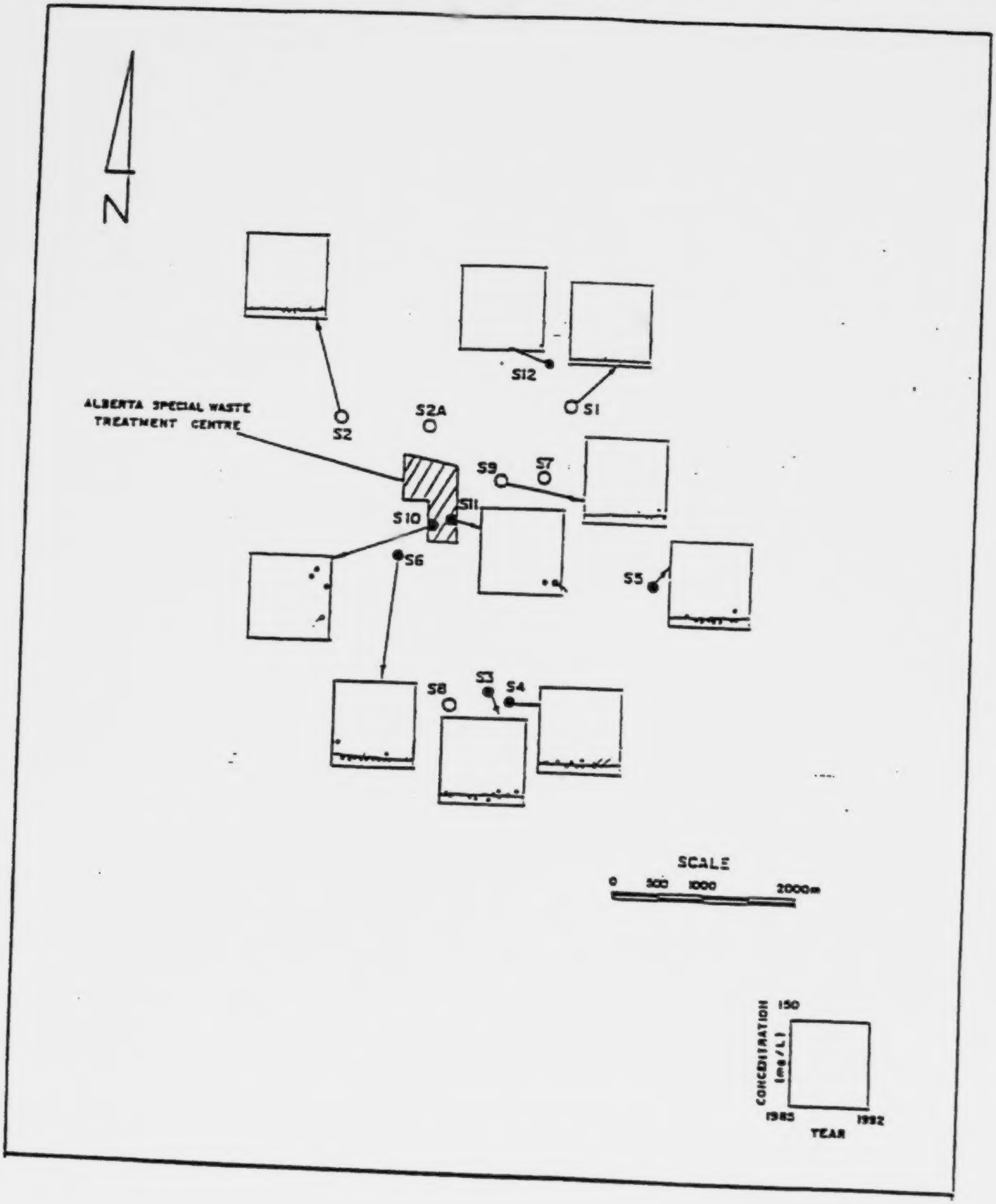

N
ALBERTA SPECIAL WASTE TREATMENT CENTRE
S12
S1
S2
S2A
S9
S7
S11
S10
S6
S5
S8
S3
S4
SCALE
0
500
1000
2000m
CONCENTRATION (mg/L)
30
1983
1992
YEAR

Figure 4. Total Organic Carbon Surface Water


N
ALBERTA SPECIAL WASTE TREATMENT CENTRE
S1
S2
S2A
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S9
S10
S11
S12
SCALE
0
500
1000
2000m
150
CONCENTRATION (mg/L)
1985
1992
YEAR

Figure 5. Chemical Oxygen Demand Surface Water

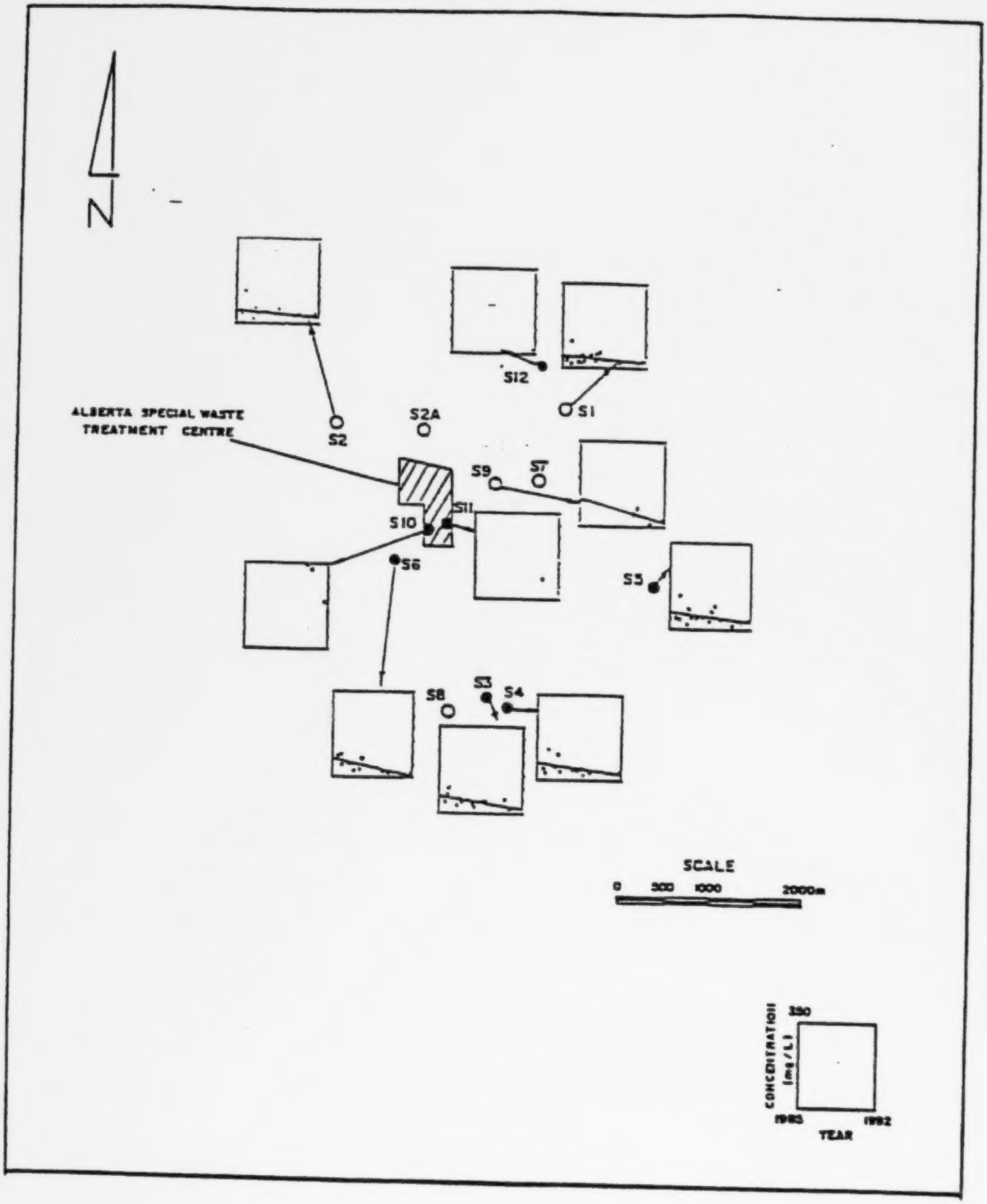
N
S12
S1
S2
S2A
ALBERTA SPECIAL WASTE TREATMENT CENTRE
S9
S7
S11
S10
S6
S5
S8
S3
S4
SCALE
0
300
1000
2000m
350
CONCENTRATION (mg/L)
1983
1992
YEAR

Table 1. 1991 Monitored Water Quality Parameters and Detection Limits

Parameter	Detection limit	Units
SPRING SAMPLING		
pH	0.1	units
Conductance	0.1	umhos/cm
Total dissolved solids	1	mg/L
Total organic carbon	0.2	mg/L
FALL SAMPLING		
pH	0.01	units
Conductance	0.1	umhos/cm
Calcium	0.01	mg/L
Magnesium	0.01	mg/L
Potassium	0.01	mg/L
Sodium	0.01	mg/L
Chloride	0.01	mg/L
Sulphate	0.01	mg/L
Nitrate	0.003	mg/L
Total dissolved solids	1	mg/L
Total organic carbon	0.2	mg/L
Chemical Oxygen Demand	0.1	mg/L
Oil and grease	0.2	mg/L
Bicarbonate	0.5	mg/L
Cyanide	0.001	mg/L
Antimony	0.0002	mg/L
Arsenic	0.0002	mg/L
Barium	0.01	mg/L
Beryllium	0.001	mg/L
Cadmium	0.001	mg/L
Chromium	0.001	mg/L
Cobalt	0.001	mg/L
Copper	0.001	mg/L
Lead	0.002	mg/L
Mercury	0.001	ug/L
Molybdenum	0.001	mg/L
Nickel	0.001	mg/L
Selenium	0.0002	mg/L
Silver	0.001	mg/L
Thallium	0.001	mg/L
Vanadium	0.001	mg/L
Zinc	0.001	mg/L
1,2-dichlorobenzene	3	ug/L
1,3-dichlorobenzene	3	ug/L
1,4-dichlorobenzene	3	ug/L
1,2,4-trichlorobenzene	3	ug/L
Hexachlorobenzene	3	ug/L
Pcb (aroclor)	0.04	ug/L

For some of the parameters in the program, very low (or non-detectable) concentrations are to be expected. PCBs, for example, will be found preferentially in sediments, as will many metals. Further, in streams the chemical composition may vary substantially with time. It may be quite expensive to measure some parameters at the low levels which might be expected in all but grossly contaminated waters. As wind direction and velocity shift, and the chemistry of the stack and fugitive emissions change, so too will the chemistry of any affected water.

CONCLUSIONS AND RECOMMENDATIONS

The Treatment Centre has had a discernible effect on surface water quality only in the area closest to the site. The site with greatest evidence of contamination, S10, appears to have been affected by a fire training site (more substantially than from normal Treatment Centre operations). Slightly elevated sulphate and chloride levels have also been observed, but well below ecologically significant levels.

It is important, however, that monitoring continue to be carried out, particularly at the two sites adjacent to the Treatment Centre. Site S11 is particularly important, as the majority of the drainage from the landfill area drains leaves the site at this point.

Given the potentially rapid changes in water chemistry which may occur in the small streams around the site, and the tendency of some of the measured parameters to accumulate in sediments, it is felt that expansion and improvement to the sediment program is warranted. Specifically, the analytical protocol should be modified so that parameters with low solubilities and high sediment sorption coefficients are analyzed in sediment rather than water. Sediment monitoring will be discussed in detail in the next section.

SEDIMENT

INTRODUCTION

Sediment samples were collected from 10 sites, shown in Figure 1. Where possible, these sites are also the water quality sampling locations. In some cases, the two locations may not coincide, as with Sites S2 and S2A. Sediment sampling is performed in conjunction with benthic monitoring in order to assess the impact of sediment chemistry on the benthic community. Further, the optimal water quality and benthic sites may not correspond.

METHODS

Sediment samples were collected from eleven specified sampling sites in streams surrounding the facility and Chrystina Lake between September 24th and October 2nd, 1991 (Figure 1). At each site, five (5) samples were taken in amber, glass jars with Teflon lids that were supplied by the laboratory, Chemex Labs Alberta Ltd.

Samples were collected from depositional areas of streams by lowering the jar in an inverted position through the water column, inserting it into the bed material and scooping fine textured sediment directly into the jars. Lake sediment samples were collected by the same technique in approximately 0.25 m of water along the shore immediately adjacent to benthic sampling sites. The labelled samples were immediately sealed, refrigerated in ice chests and returned to Calgary for laboratory analysis.

The five (5) sediment samples from each site were composited by Chemex Labs and analyzed for polychlorinated biphenyls (PCBs) (Hexane extraction and EPA 8000A and 8080A) and chlorinated benzenes screen (EPA 8270).

RESULTS AND DISCUSSION

Results of the analyses for PCBs and chlorinated benzenes in sediment are presented in Table 2.

Polychlorinated biphenyls at or slightly above the practical quantification limit (PQL 0.01 µg/g), were found, for the first time, in sediment samples from nine sites. Concentrations below the PQL were found at sites S3, south east of the facility, and S10, the west, on-site drainage ditch.

The maximum PCB concentration, 0.05 µg/g, was found at sites S2A, immediately north of the facility and S5, 2 km. east south east, the predominant downwind direction. It should be emphasized; however, that in previous annual assessments the detection limit for PCB analysis of sediment was 0.05 µg/g. The two highest concentrations found in 1991 would have been the only sites where PCB could possibly have been detected using the previous detection limits.

In previous years, no chlorinated benzenes were detected. However, improved detection limits in 1991 led to the discovery of 1,4-dichlorobenzene (DCB) in sediment for the first time at all sites sampled. Trace quantities above the detection limit of 0.033 µg/g but less than the quantification limit of 0.099 µg/g were found at S7 and S9 immediately east of the facility and S8 directly south. Concentrations elsewhere ranged from a low of 0.118 µg/g at site S6 southwest of the facility to a maximum of 0.339 µg/g in Chrystina Lake (S12) sediment. No other dichlorobenzene isomers were detected in sediment samples.

1,2,4-trichlorobenzene (TCB) was detected in trace quantities (<0.099 µg/g) in the on-site drainage ditches and Chrystina Lake.

In previous monitoring reports halogenated organic compounds were not detected in sediment. The detection limit for this program (0.033 µg/g) was lower than in the past (0.05 µg/g) but some of the concentrations reported in this program were substantially higher than the previous detection limit. There was no apparent pattern of chlorobenzene concentrations that was consistent with prevailing winds or with PCB results. Only trace concentrations were found east of the facility but the highest concentration was found in Chrystina Lake sediment which was further from the facility to the north east.

PCBs and chlorinated benzene were not detected in the method or instrument blanks. Furthermore, all other quality control and calibration checks were within Chemex's QA/QC acceptability criteria.

Table 2. 1991 Sediment Chemistry Results

SITE	PCBs (AROCLOR) ug/g	1,2 - DI-CHLOROBENZENE ug/g	1,3 - DI-CHLOROBENZENE ug/g	1,4 - DI-CHLOROBENZENE ug/g	1,2,4 - TRI-CHLOROBENZENE ug/g	HEXA - CHLOROBENZENE ug/g
S2A	0.05	NOT DETECTED	NOT DETECTED	0.128	ND	NOT DETECTED
S3	ND			TR	ND	
S4	0.04			0.208	ND	
S5	0.05			0.259	ND	
S6	0.02			0.118	ND	
S7	0.04			TR	ND	
S8	0.03			TR	ND	
S9	0.01			TR	ND	
S10	ND			0.204	TR	
S11	0.04			0.122	TR	
S12	0.03			0.339	TR	
Detection Limit (ug/g)	0.04	0.033	0.033	0.033	0.033	0.033
Quantitation Limit (ug/g)	0.01	0.099	0.099	0.099	0.099	0.099

TR = trace (less than quantitation limit)
ND = not detected

The concentrations of PCBs found in the 1991 sediment sampling program were not alarming compared to studies conducted elsewhere. PCBs concentrations of 0.02 and 0.6 µg/g have been reported in surficial sediments of Lake Erie and concentrations as high as 2.7 µg/g were reported in Niagara River sediment (CCREM 1991). These elevated levels were attributed to point source discharge to water bodies from industrial activity and sewage treatment plants. Considerably lower concentrations are expected from non-point sources where dispersion is expected to occur during transport.

The Aroclor identified, 1260, is a mixture of many biphenyls with varying degrees of chlorination. It has a low water solubility, 0.080 mg/L at 24°C and a relatively high sediment sorption coefficient (log K_{oc} = 6.42 estimated) and octanol/water partition coefficient (log K_{ow} = 6.91) (Montgomery and Welkom 1990). As such, Aroclor 1260 will tend to have a high affinity for sediment, particularly organic sediments, and tend to accumulate in the fatty tissue of aquatic organisms. Bio-concentration factors of 200,000 to 1,000,000 have been reported (CCREM 1991) and therefore relatively low concentrations in sediment can result in high PCB levels in biota. PCBs are extremely resistant to oxidation and hydrolysis and the rate of biodegradation decreases with the degree of chlorination. PCB degradation products include benzoic acid, chlorobenzoic acid, chlorobiphenyldiol and other aliphatic and aromatic hydrocarbons (CCREM 1991).

Chemex Laboratories was asked to confirm the chlorinated benzenes that had been detected in sediment samples. As DCB or TCB were not detected in either the method blank or instrument blank, Chemex was confident that the positive results were not due to the methodology, solvent or instrumentation used. Chemex also emphasized that the response factor used in the sediment analysis set the quantification limit at 0.099 µg/g. The USA EPA quantitation limit for this technique is usually 0.330 µg/g and all concentrations found were very near or below this limit (B. Swingle, pers. comm., 1991). While the chlorobenzene results cannot be referred to as "false positives" or "laboratory artifacts", they are very low concentrations near the practical limits of this technique.

According to references cited in the Canadian Water Quality Guidelines (CCREM 1991), chlorinated benzenes are ubiquitous in the aquatic environment and have been detected in water, sediment and aquatic biota. They are relatively hydrophobic in nature (water solubility 83.2 mg/L, CCREM 1991), have a moderate sediment sorption coefficient (log K_{oc}: 2.20, Montgomery and Welkom 1990) and high octanol/water coefficient (log P_{ow}: 3.37, CCREM 1991). As a result, chlorinated benzenes tend to sorb to sediment particles. Concentration of chlorinated benzenes in lake sediments has been demonstrated. Chlorinated benzenes are lipophilic in nature, expected to accumulate in aquatic organisms and bioconcentration is possible (log BCF 2.57 ± 1.86, CCREM 1991). They do not appear to metabolize or degrade easily and resistance to microbial degradation increases with the degree of chlorination.

1,4-dichlorobenzene or *para*dichlorobenzene is used in the preservation of furs and woolens and as a killing agent for moths. It's most common use is a bathroom deodorant where it is typically found in urinal blocks. 1,4 dichlorobenzene is federally registered for use as a pesticide in Canada (G. Byrtus pers. comm., 1992) but its primary use is as an indoor moth killer.

1,2,4-trichlorobenzene is used as a solvent, dye-carrier, herbicide/insecticide intermediate, transformer fluid, lubricant and degreaser. (Montgomery and Welkom 1990, CCREM 1991). It is used as an herbicide in the USA for control of aquatic vegetation but has not been registered for use in Canada (G. Byrtus pers. comm., 1992).

The chlorinated benzenes found in this program have not been found as impurities or degradation products of pesticides commonly used in Alberta. If these were pesticide degradation products the chemical structure of these materials would suggest that other isomers would be present as well.

CONCLUSIONS AND RECOMMENDATIONS

The ubiquitous nature of PCBs found in the 1991 survey was noteworthy and temporal trends should be closely monitored. As PCBs are bio-accumulated, monitoring in sediment at detection limits capable of discerning ecologically significant concentrations is essential.

1,4-dichlorobenzene and 1,2,4-trichlorobenzene were detected in very low concentrations at or below the usual USA EPA detection limit guidelines for these analytical techniques. Had the higher detection limit been used, these chlorinated benzenes would not have been detected. Continued monitoring for these chlorinated benzenes in sediment is recommended to discern temporal trends.

The detection of PCBs in sediment confirms the results from other components of the monitoring program. Air quality, animal, and vegetation monitoring have all identified the presence of PCBs around the site. Waste handling procedures have been improved to reduce fugitive emissions (Chem-Security, 1991), which appear to be the primary source of PCBs.

It is essential to ensure that the analytical protocols used are sufficient to detect the levels of contamination expected. Ecologically significant concentrations of the parameters of concern are being defined. Analytical techniques are being investigated that will ensure the instrument detection limit is three times lower than those concentrations. Guidelines for the Protection of Freshwater Aquatic Life (CCREM 1991) are recommended for freshwater and similar USA and Ontario M.O.E. guidelines for sediment are being assessed.

The sediment program is being modified to ensure more accurate assessment and greater comparability between sites. The sampling protocol is being modified slightly to ensure only recently deposited material is sampled. Sediment grain size effects will be minimized by sieving to ensure collection of only fine sediment. The grain size distribution of a subsample of the material actually analyzed will then be determined. The organic matter content of the sediment will also be assessed and the necessary corrections made.

Lastly, the quality assurance program has also been improved by utilizing standard reference materials of known chemical composition.

ACKNOWLEDGEMENTS

The authors wish to thank Chem-Security (Alberta) Ltd., operators of the Alberta Special Waste Treatment Centre in Swan Hills, for their encouragement in the writing of this paper, and their permission to release the information contained within.

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CHEMICAL CHARACTERIZATION, AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF UNTREATED EFFLUENT DISCHARGES FROM THREE TEXTILE MILLS IN THE ATLANTIC REGION. L.A. Rutherford, W.R. Ernst, K.G. Doe and P.A. Hennigar, Environment Canada, Conservation and Protection, Atlantic Region, Dartmouth, N.S., (902) 426-6141.

SUMMARY

At four times during the fall of 1990, final effluent samples were collected for chemical characterization and toxicity assessment from the three textile mills surveyed. GC/MS/Computer techniques were used to chemically characterize organic components of mill effluent and to quantify a limited number of target compounds in environmental samples collected near mill effluent outfalls. In addition, a number of conventional pollutant parameters such as pH, BOD, COD, TSS and metals were quantified in effluent samples. Rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna* and *Photobacterium phosphoreum* (Microtox) tests were conducted on 12 effluent samples to determine the acute lethality of the effluent. Two samples from each mill were tested for sub-lethal toxicity to *Selenastrum capricornutum* and *Ceriodaphnia dubia* and for genotoxicity (Ames test).

Using artificial substrate samplers, benthic macroinvertebrate abundance was determined at five stations (one control, four impacted stations at various distances from the effluent outfall) in the receiving environments. In order to measure the accumulation of contaminants in the aquatic environment at one of the mills, caged freshwater clams (*Anodonta implicata*) were deployed at each of the five sampling stations during the fall of 1990 and spring of 1991.

Effluent collection, chemical characterization, aquatic toxicity testing and environmental field sampling methods used in the study are described in detail in Rutherford et al. (1992).

Organic chemicals identified in effluent samples from the textile mills studied generally fell into one of five groups: detergents/surfactants (e.g. ethoxy and phenoxyethanols, ethylhexanol, nonylphenol); plasticizers (e.g. diethylphthalate, bis(hexylethyl)phthalate); dye carriers (e.g. alkylated benzenes, mono-, di- and tri-methylnapthalenes, biphenyl, methylbiphenyl, benzoic acid); mineral oils (e.g. C10-C32 n-alkanes); and miscellaneous chemicals (e.g. methylpyrolidinone, caprolactum).

The greatest number of compounds identified in the effluent samples were typical of auxiliary chemicals required for satisfactory dyeing. Of these, dye carriers made up the largest portion of all compounds identified. The USA EPA (1978) estimated that 90 percent of the dye carriers used in the dyeing process are consumed in the operation with the remaining 10 percent being rinsed to waste.

Of the twelve chemicals monitored in clams held within Mill B's outfall, 1-methylnaphthalene (1.0 ug/g), 2-methylnaphthalene (1.2 ug/g), biphenyl (2.8 ug/g), 1,2-dimethylnapthalene (0.2 ug/g), 1,4-dimethylnapthalene (0.7 ug/g), 1,6-dimethylnapthalene (2.1

ug/g) and 2,3,5-trimethylnaphthalene (0.1 ug/g) were detected above the method detection limit of 0.1 ug/g wet weight in clams held 120 m downstream of the mill's outfall. All of those chemicals are dye carriers. No surfactants, such as 2-butoxyethanol or 2-ethylhexanol, were detected in the clam samples. No target compounds were detected in the control sample of clams held 85 m upstream of the mill's effluent outfall.

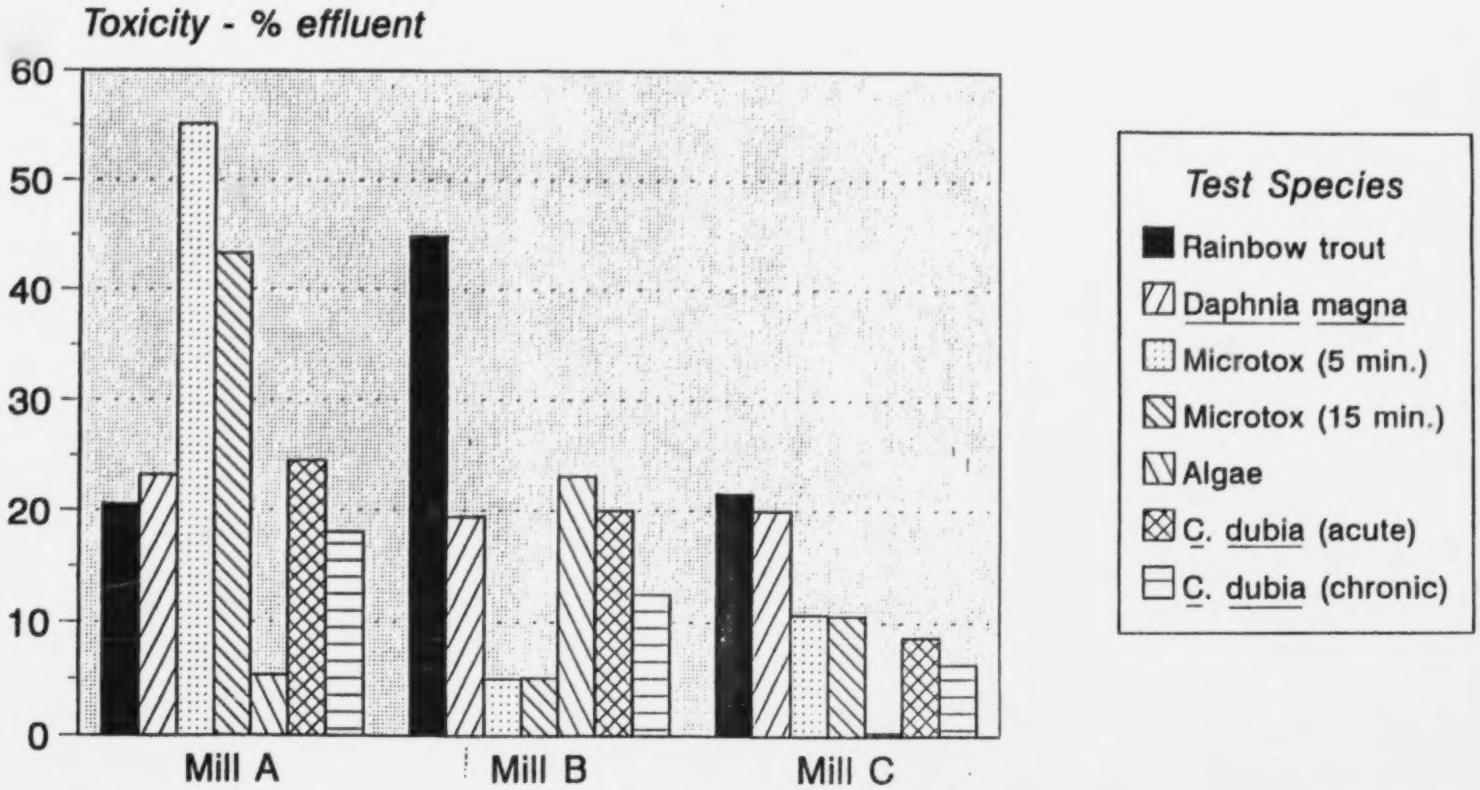
With respect to conventional pollutants, textile wastewaters are typically characterized by extreme pH, elevated temperatures, and high concentrations of BOD, COD, total suspended solids and heavy metals (Netzer and Beszedits 1975; Chen 1989). In most of the effluent samples from the mills studied, pH was within the range of 6 to 9 that has been measured in previous Environment Canada surveys of textile mills in Canada (Chen 1989). BOD levels ranged from 13 mg/L to 350 mg/L in ten of twelve samples, thereby exceeding a proposed maximum permissable discharge limit direct to the environment of 20 mg/L BOD (NSDOE 1989). Effluent samples were 10 to 36 times higher than the proposed discharge limit of 30 mg/L for COD. In Environment Canada and USA EPA surveys, zinc, copper and chromium were the most frequently found metals in textile effluent in concentrations ranging from 0.1 mg/L to 0.2 mg/L (Chen 1989). The highest concentration of metals found in effluent in this study was a sample that had 0.17 mg/L zinc. Eleven of twelve samples had these metals in concentrations well below 0.1 mg/L.

All samples collected from the three textile mills surveyed were acutely toxic to all organisms tested, except one sample from Mill A in the Microtox test (Figure 1). All samples had sub-lethal effects on all species tested including reproductive impairment in *Ceriodaphnia dubia* and growth impairment in the alga *Selenastrum capricornutum*. The untreated effluent was moderately toxic to rainbow trout with LC50 values ranging from 11.5% to 35.4% effluent. One sample from Mill C was highly toxic with a LC50 of 8.2% effluent. These values are comparable to acute toxicity results reported in previous Environment Canada studies for textile mills with primary treatment (Chen 1989). The high toxicity of Mill C samples and the most toxic Mill A sample to the alga *Selenastrum capricornutum* suggested the possibility of biological effects in the receiving environment at concentrations as low as 0.1% effluent. In this study, algae were the most sensitive to textile mill effluent, an observation which has also been made by others (Walsh et al. 1980). The *Ceriodaphnia dubia* IC50 values ranged from 1.8% to 8.7% effluent. With reproductive impairment at such low concentrations of effluent, a disruption of energy flow in the receiving waters of these mills would be a possibility.

Ames testing indicated that all samples were mutagenic (Figure 2). There were differences in the apparent type of mutagenicity associated with each sample. For instance, only one sample from Mill C caused mutagenicity in the bacterial strain TA100, while other samples were mutagenic to one or more tester strains. The results suggest that more than one mutagen is present in the samples. The disappearance rate of those mutagenic compounds in the receiving environments is a significant unanswered question.

Benthic macroinvertebrate surveys at Mill B indicated that untreated effluent discharged from the mill caused a biological impact in the receiving environment. Species diversity, as measured by the mean number of taxa at the sampling stations, was significantly lower at all of the impacted stations compared to the control during both field surveys (Dunnett's T test $p<0.05$). In the fall of 1990, 13 of 14 taxa statistically analyzed had significantly lower numbers at the impacted stations compared to the control ($p<0.05$). In five of seven taxa statistically analyzed from the spring of 1991 sampling run, a significant decrease in numbers was observed at three or more of the impacted stations ($p<0.05$).

Figure 1. **Toxicity of Untreated Effluent from Three Textile Mills**



(Toxicity - mean of LC50 or EC50 data for all dates)

Figure 2. Mutagenicity of Untreated Effluent from Three Textile Mills

Sample location and date	*Tester strain TA97*	*Tester strain TA98*	*Tester strain TA100*	*Tester strain TA102*
Mill C - Sept. 12/90	*S - P*		*S - P*	
Mill A - Sept. 19/90				*S - P*
Mill C - Sept. 26/90		*P*		*S - P*
Mill A - Oct. 3/90	*P*	*S - P*		*S*
Mill B - Oct. 10/90		*P*		*S - P*
Mill B - Oct. 31/90	*S - P*			*S - P*

Mutagenesis detected using the Ames Spot test (S) and the Plate Incorporation test (P).

Aquatic insects, snails and leeches were all negatively impacted. In three of four taxa collected during both surveys, a significant reduction in abundance of individuals was observed at impacted stations in both fall and spring.

At Mill B, where freshwater clams were deployed in cages, all of the clams survived at the control station during both surveys, whereas all clams died in the effluent plume in the fall of 1990 and all clams but four (at Station 4 - 120 m from outfall) died during the spring of 1991 survey.

Based on the results of this study, the following recommendations are made.

1. Untreated textile effluent should not be discharged directly to a watercourse or to a municipal system without wastewater treatment.
2. Given the complex chemical nature of textile effluent, and the fact that a number of constituents of textile effluent may be toxic to aquatic organisms, a battery of toxicity tests should be used to assess the toxicity of textile wastewaters.
3. Regulatory control of wastewater discharges from textile mills is required.
4. The overall adequacy of effluent regulations should be assessed by monitoring for environmental effects in receiving environments.

The following future studies are required:

1. An evaluation of the toxicity and environmental impact of treated textile effluent discharges on freshwater ecosystems.
2. An identification of organic indicator/target compounds in textile effluent based on chemicals used in textile processes and chemical characterization of treated effluent.
3. An examination of the fate and effects of dyes in the aquatic environment downstream from textile mills.
4. An identification of the mutagenic compounds in textile effluent discharges and the determination of their disappearance rates in receiving environments.

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AQUATIC TOXICITY REDUCTION AT EIGHT MUNICIPAL WASTEWATER TREATMENT PLANTS IN ALBERTA.

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ABSTRACT

Reduction in the acute toxicity of wastewater from eight municipal treatment plants in Alberta was determined using rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), a cladoceran (*Daphnia magna*), and a mollusc (*Anodonta grandis*). The plants represented three basic design types: lagoon, aerated lagoon, and mechanical-biological. Prior to implementation of toxicological procedures, the wastewater samples were manipulated to reduce ammonia-N concentrations. All plants were successful in reducing toxicity, regardless of the time of year. Effluent from the plants was non-acutely toxic to the four species but influent was toxic to at least one species in 75% of the plants. Calgary and Red Deer, both mechanical biological plants, had no mortality in their influent. *Anodonta grandis* was the most sensitive test species to influent from three of the eight plants, followed by fathead minnow (two plants) and rainbow trout (one plant). No mortality was observed following exposure to influent from two plants. Histopathological evaluation of fish exposed to influent from all plants revealed mild to moderate lesions associated with the gill lamella and skin. The frequency of these lesions was relatively low in effluent-exposed fish.

Introduction

Municipal wastewater treatment plants are designed to reduce the toxicity of many agents prior to discharge to surface waters. Plant performance varies with the basic plant design, waste, season, and maintenance of the treatment facilities. Hannah et al. (1986), for example, showed that the effectiveness in removal of heavy metals was approximately similar among activated sludge, aerated lagoons, and facultative lagoons. Primary clarification, on the other hand, was least effective, followed by trickling filter. Organic compounds were most efficiently removed by facultative lagoon and activated sludge.

If the type of treatment system is not designed to remove specific compounds, toxic wastewater may be produced. For example, a study of plants in the Province of Ontario (Canada) demonstrated that toxicity of the effluent was generally due to high concentrations of ammonia-N, and poor BOD removal (Ontario Ministry of Environment 1990).

The purpose of this study was to determine the reduction in toxicity at eight major municipal treatment plants situated in Alberta, Canada. Acute toxicity was determined using rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), a cladoceran (*Daphnia magna*), and a mollusc (*Anodonta grandis*). Histopathologic evaluation was conducted on fish exposed to both influent and effluent.

Materials and Methods

Municipal Wastewater Treatment Plants

The eight municipal wastewater treatment plants were located in Calgary (Bonnybrook plant), Grande Prairie, Fort McMurray, Camrose, Wetaskiwin, Lethbridge, Medicine Hat, and Red Deer (Table 1). During this study, Camrose used a lagoon treatment system, whereas Fort McMurray and Wetaskiwin used aerated lagoons. The Medicine Hat plant also used an aerated lagoon system prior to the spring (1990) but then switched to mechanical-biological treatment. All other plants also used a mechanical-biological system.

Collection Procedures and Sample Manipulation

Samples were collected from each plant during warm weather and cool weather operating conditions (Table 2). All influent samples were collected immediately after the grit screens whereas all effluent samples were taken immediately prior to discharge to the receiving waters.

Table 1. Municipal wastewater treatment methods used at the eight plants.

Calgary (Bonnybrook)
Screening> grit removal> primary clarification> aeration (activated sludge)> secondary clarification and phosphorus removal (alum precipitation)> continuous discharge (Bow River).
Camrose
Wastewater stabilization ponds (four anaerobic cells, one facultative cell, and four storage cells - seven months storage)> discharge twice per year (spring and fall to Battle River via Camrose Creek).
Fort McMurray
Aerated lagoons> continuous discharge (Athabasca River).
Grande Prairie
Screening> grit removal> primary clarification> biological treatment (rotating biological contact process)> secondary clarification> storage lagoon (30 days)> continuous discharge (Wapiti River).
Lethbridge
Mechanical screening> grit removal> primary clarification> aeration (activated sludge)> secondary clarification> continuous discharge (Oldman River). Note: plant consists of two parallel plants one for domestic wastewater treatment and one for industrial wastewater treatment. Final discharge is a mixture of effluents from the two plants.
Medicine Hat
Prior to March (1990): Grit removal> primary clarification> aeration (four aerated lagoons)> facultative lagoon> continuous discharge (South Saskatchewan River). Note: some wastewater irrigation).
After March (1990): Grit removal> primary clarification> biological treatment (trickling filter-solids contact process)> secondary clarification with phosphorus removal> disinfection using Cl_2> polishing and dechlorination ponds> continuous discharge (South Saskatchewan River).
Red Deer
Screening> grit removal> primary clarification> aeration (activated sludge)> secondary clarification> continuous discharge (Red Deer River).
Wetaskiwin
Screening> aerated lagoons> polishing ponds> storage lagoon (seven months)> discharge, twice per year (Battle River via a tributary).

Table 2. Collection dates for samples used in acute toxicity tests.

PLANT	SAMPLING DATES
Calgary (Bonnybrook)	January 18, 1990; June 6, 1990
Camrose	November 16, 1989; May 3, 1990
Fort McMurray	October 6, 1989; March 1, 1990
Grande Prairie	October 12, 1989; January 4, 1990
Lethbridge	February 8, 1990; May 31, 1990
Medicine Hat	January 12, 1990; May 24, 1990
Red Deer	December 7, 1989; June 21, 1990
Wetaskiwin	November 2, 1989; April 24, 1990

Unlike all other systems, the Lethbridge plant had two parallel processes, one for treatment of domestic wastewater and the other for treatment of industrial wastewater (Table 1). The influent samples used for toxicity evaluation were obtained by manually combining equal amounts of wastewater from the two processes. On the other hand, the effluent from the two processes was mixed within the plant as part of the treatment process prior to discharge. Hence there was no need to manually combine wastewater from the two processes.

Grab samples were collected in a 10-L stainless steel pail and placed in 20-L polypropylene containers. A total of 120 L of both influent and effluent was collected on each sampling date. All samples were taken over a relatively short time period (1-2 min.), so potential daily (or longer term) changes in hydraulic flow and wastewater quality may have gone undetected by the sampling regime. However, since the toxicological procedures used in this study yielded consistent data (see Results), the extent of diurnal changes in the toxicological properties of the wastewater was probably minimal.

During transport, the samples were held at ambient temperature. On receipt at the laboratory influent and effluent samples were poured into individual 300-L polypropylene tanks. The sample temperature, pH, conductivity, and dissolved oxygen were then determined using a Hydrolab meter. A 500-mL aliquot was taken for analysis of heavy metals (Al, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Se, Zn), preserved by acidification, and analyzed by Chemistry Division using standard methods (Alberta Environmental Centre 1987). Because of the comprehensive nature of the data generated, only summaries of these findings are given in the Results.

Preliminary analysis indicated that the concentration of ammonia-N in several samples was high and might result in rapid death of experimental organisms. Because the toxicity of ammonia-N had already been well established by other investigators, the samples were manipulated using the following procedure to reduce ammonia-N. All samples were aerated for 48 hours to raise pH to approximately 8.5. At this pH, ammonia-N dominates the ammonia/ammonium complex, and results in the volatilization of free ammonia. pH was then adjusted to 6.0 using 1-10 N HCl to reduce the proportion of residual ammonia-N to <0.01%. The samples were then poured for implementation of toxicologic procedures. A 125-mL aliquot was taken and cooled to 4°C for approximately 24 hours prior to analysis of ammonia-N. The analysis was conducted by a commercial laboratory (Norwest Labs, Edmonton) using an automated colorimetric method. It is recognized that these manipulative procedures may have amended the samples in ways other than the removal of ammonia-N. However, the concentration of ammonia-N in samples that were not manipulated was so high that all test species would have died in a relatively short time period (particularly in the influent). Manipulation of samples makes possible the evaluation of toxicity not related to ammonia-N.

Toxicologic Procedures

Rainbow trout, fathead minnow, *Daphnia magna*, and *Anodonta grandis* were acquired and maintained according to Standard Operating Procedures (Aquatic Biology Branch, 1991). A summary of water quality conditions during maintenance is in Table 3. All acute toxicologic procedures involving the species were implemented using Standard Operating Procedures (Aquatic Biology Branch, 1991). Because influent samples were often highly turbid, it was not possible to implement the *Daphnia magna* test on such material. Histopathologic evaluation was conducted on tissues of a representative number of fish exposed to wastewater from each plant. Approximately five fish were taken from each exposure chamber at the end of the experiment, fixed in Bouin's solution, and preserved in formalin. All other fish were killed with an overdose of MS222. Because of resource restraints, it was not possible to conduct histopathologic on all fish from all samples. Examples of water quality conditions in the test chambers during implementation of the above-noted toxicologic procedures are listed in Tables 4a and 4b.

Table 3. Summary (average, range) of water quality conditions used during the maintenance of the four test species.

SPECIES	TEMPERATURE (°C)	DISSOLVED OXYGEN (mg/L)	CONDUCTIVITY (µS/cm)	pH
Rainbow trout	12.9 (10.1-15.0)	10.6 (9-13)	222 (200-280)	7.7 (7.0-8.3)
Fathead minnow	22.1 (13.6-28.0)	7.1 (4.8-8.3)	289 (251-645)	8.0 (7.1-9.7)
Daphnia magna	20.0 (18.4-21.3)	7.1 (4.8-8.3)	596 (324-727)	8.3 (7.4-9.5)
Anodonta grandis	21.0 (17.6-22.9)	7.3 (6.3-9.6)	570 (298-877)	8.3 (7.8-8.6)

Table 4a. Summary (average, range) of water quality conditions in control chambers during implementation of toxicological procedures.

PROCEDURE	TEMPERATURE (°C)	DISSOLVED OXYGEN (mg/L)	CONDUCTIVITY (µS/cm)	pH
96-h LC_{50} rainbow trout	14.8 (12.7-20.6)	8.7 (7.4-9.7)	292 (274-316)	7.5 (6.1-8.1)
96-h LC_{50} fathead minnow	20.4 (18.7-22.5)	7.8 (6.6-9.0)	296 (274-322)	7.6 (6.5-8.5)
96-h LC_{50} *Anodonta grandis*	18.5 (13.0-22.1)	8.2 (7.2-10.5)	309 (208-361)	7.6 (5.8-8.3)
48-h LC_{50} *Daphnia magna*	20.5 (18.5-22.3)	7.9 (7.0-9.1)	506 (254-670)	8.1 (7.0-8.7)

Table 4b. Summary (average, range) of water quality conditions in undiluted (100%) wastewater during implementation of toxicologic procedures.

PROCEDURE	TEMPERATURE (°C)	DISSOLVED OXYGEN (mg/L)	CONDUCTIVITY (µS/cm)	pH
96-h LC_{50} rainbow trout	15.1 (12.7-20.5)	8.6 (7.0-9.8)	1386 (827-2330)	7.5 (5.9-8.1)
96-h LC_{50} fathead minnow	20.2 (19.2-22.5)	7.5 (4.0-9.3)	1563 (954-4020)	7.4 (5.9-8.5)
96-h LC_{50} *Anodonta grandis*	18.5 (16.6-23.7)	7.9 (3.4-9.9)	1523 (840-4300)	7.5 (5.1-8.3)
48-h LC_{50} *Daphnia magna*	20.6 (18.7-22.0)	7.5 (4.6-8.6)	1272 (403-2200)	7.1 (5.8-8.2)

Results

Chemical Analysis

Of the 15 metals (Al, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Se, Zn) analyzed in the wastewater samples, the majority underwent a moderate to substantial decline in concentration after passing through the treatment plants. Examples of water analysis of important parameters for the three plant designs (lagoon, aerated lagoons, mechanical-biological) are listed in Tables 5-7. Removal efficiencies generally fell in the 50-95% range for most metals. Only Aluminum, Copper and Zinc routinely exceeded national and international guidelines for the protection of fish. Greatest Aluminum concentrations were found in the influent of the Fort McMurray plant (1.462-5.951 mg/L), followed by Camrose (0. 149-4.715 mg/L). Three cities (Calgary, Grande Prairie, Medicine Hat) added alum to clarify the wastewater, resulting in elevated concentrations of Aluminum in the effluent.

Table 5. Chemical analysis (mg/L) of some important water quality parameters in municipal wastewater collected from Camrose (lagoon system).

DATE	NOVEMBER 16, 1989		MAY 3, 1990	
PARAMETER	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.149	0.033	4.715	0.157
Arsenic	0.0021	0.0064	0.004	0.0031
Cadmium	0.004	0.003	0.003	0.003
Chromium	0.008	0.003	0.011	0.005
Copper	0.155	0.003	0.164	0.007
Lead	0.069	<0.002	0.364	<0.002
Zinc	0.240	0.006	0.215	0.016
Ammonia-N	29.0	0.300	27.1	21.7

Table 6. Chemical analysis (mg/L) of some water quality parameters in municipal wastewater collected from Fort McMurray (aerated lagoon system).

DATE	OCTOBER 6, 1989		MARCH 1, 1990	
PARAMETER	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	5.951	0.138	1.462	0.127
Arsenic	0.0048	0.0018	0.0012	0.0007
Cadmium	0.002	0.001	0.002	0.001
Chromium	0.005	0.002	0.005	0.003
Copper	0.043	0.004	0.052	0.004
Lead	<0.002	<0.002	0.017	<0.002
Zinc	0.103	0.019	0.181	0.066
Ammonia-N	21.0	0.300	45.0	26.0

Table 7. Chemical analysis (mg/L) of some important water quality parameters in municipal wastewater collected form Grande Prairie (mechanical-biological system).

DATE	OCTOBER 12, 1989		JANUARY 4, 1990	
PARAMETER	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.770	0.062	0.468	0.339
Arsenic	0.0008	0.0010	0.0015	0.0001
Cadmium	0.003	0.002	0.002	0.002
Chromium	0.004	0.002	0.003	0.002
Copper	0.034	0.006	0.041	0.019
Lead	0.007	0.002	0.006	<0.002
Zinc	0.077	0.043	0.060	0.065
Ammonia-N	72.8	0.069	26.9	13.1

Acute Toxicity

Influent from the plants was acutely toxic in several instances (Table 8). Of the six tests completed on influent from Fort McMurray, four were toxic. Similarly, the Grande Prairie, Medicine Hat, and Wetaskiwin plants produced two cases of acute mortality each. *Anodonta grandis* was the most sensitive test species in three of the eight plants, followed by fathead minnow (two plants) and rainbow trout (one plant). No mortality was observed at two plants (Calgary, Red Deer).

In all cases but one, effluent from the eight plants was non-acutely toxic (LC_{50}s ≥100%) to the four test species. The exception was *Anodonta grandis* with an LC_{50} of 56.1% when exposed to effluent collected on March 1, 1990 from Fort McMurray.

Table 8. Acute toxicity (LC_{50}) of municipal wastewaters (influent) to rainbow trout, fathead minnow, and *Anodonta grandis*.

CITY/SPECIES	RAINBOW TROUT	FATHEAD MINNOW	*ANODONTA GRANDIS*
Calgary	≥100%	≥100%	≥100%
Camrose	92.8 - 100%	≥100%	≥100%
Fort McMurray	80.0 - 100%	69.3 - 76.3%	50 - 100%
Grande Prairie	≥100%	≥100%	77.1 - 84.1%
Lethbridge	≥100%	94.5 - 100%	≥100%
Medicine Hat	84.1 - 100%	≥100%	70.7 - 100%
Red Deer	≥100%	≥100%	≥100%
Wetaskiwin	56.3 - 100%	42.2 - 100%	≥100%

Histopathology

Mild gill and skin lesions were seen in most rainbow trout and fathead minnow exposed to influent from all of the plants except the Camrose lagoon. The most common conditions of the gills were: i) lamellar, epithelial cell hypertrophy, and ii) atrophy, blunting and edema of the gill lamella. These lesions were found in fish exposed to high concentrations (60-100%) of influent. The main lesions of the skin in both rainbow trout and fathead minnow were: i) epithelial cell hypertrophy, and ii) hyperplasia and hypertrophy of mucous cells. This would produce more mucus on the fish body, presumably for protection. Again, these conditions were generally limited to fish exposed to high concentrations of influent. No lesions were observed in rainbow trout and fathead minnow exposed to influent from the Camrose lagoon.

No lesions were usually seen in fish exposed to effluent from Camrose, Lethbridge, Red Deer and Wetaskiwin. The lesions that were observed in fish from the other municipalities were generally limited to the gills: i) mild atrophy, blunting and edema of the gill lamella, and ii) mild lamellar epithelial cell hypertrophy. Occasional disorders of the skin, such as mucous cell hyperplasia, were also noted, but only at high effluent concentrations.

Discussion

All of the plants discharged wastewater that was non-acutely toxic to the test species. Although some lesions were seen in the gills and skin of fish, these effects were mild, and, in the case of mucous cell hypertrophy, resulted in a protective effect for the fish. All three system types (lagoon, aerated lagoon, mechanical-biological) were successful in reducing toxicity, regardless of the northern latitude (and hence low winter temperature) of the systems.

Effluent from lagoon-type systems is often reported to be non-acutely toxic, particularly under summer operating conditions. This trend has been observed in Ontario, British Columbia, Saskatchewan, and a number of other localities (Metikosh et al. 1980; Ontario Ministry of Environment, 1990). Winter operating conditions present a different condition, especially if the lagoon becomes anaerobic. Hydrogen sulphide, methane, and ammonia-N may be produced, principally in the sediments, and can be trapped under the ice. This produces a toxic wastewater, particularly as the winter progresses. These effects were not noted in the current study.

Effluents from secondary treatment biological-mechanical plants are also generally non-acutely toxic, unless high ammonia-N concentrations or poorly treated industrial wastes are discharged (Metikosh et al. 1980). For example, Wylie et al. (1990) showed that fathead minnow (*Pimephales promelas*) and Cladoceran (*Ceriodaphnia dubia*) did not survive for more than a few hours in effluent from two treatment plants in Joplin, Missouri. This toxicity, a result of incomplete treatment, was due to relatively high concentrations (0.13-0.97 mg/L) of pentachlorophenol from a wood-preservative plant.

The influents of this study, although acutely toxic to at least one species in 75% of the plants, did not induce massive mortality in any species, or substantial histopathological effects in the two fish species. Although we do not have detailed chemical composition data for the influents, heavy metal concentrations were relatively low. The highest concentration of ammonia-N in an influent, 72.8 mg/L at Grande Prairie, was reduced to 0.069 mg/L in the effluent. Under typical test conditions of this study (temperature 20°C, pH 7.5), the percentage of unionized ammonia (the highly toxic form) is 1.2 of total ammonia (Piper et al. 1982). This gives a maximum concentration of unionized ammonia of only 0.9 mg/L in the influent. The LC$_{50}$s for rainbow trout and fathead minnow exposed to this concentration were both ≥100%, whereas the LC$_{50}$s for *Anodonta grandis* ranged from 77 to 84%. By contrast, Neiheisel et al. (1988), working on six treatment plants in Ohio, noted that all plant influents were highly toxic to fathead minnow and *Ceriodaphnia dubia*. However, the plant receiving the most toxic influent showed the largest reduction in toxicity.

Anodonta grandis was the most sensitive of the three test species exposed to influents from the treatment plants. This species is a filter feeder which relies on cilia on the gills to transport food to the mouth. If suspended solid loads are unduly high, the cilia and gills clog with particles, resulting in suffocation of the mollusc. Since *Anodonta grandis* showed no tendency to close its valves during our study, thereby preventing the flow of water into the mantle cavity, mortality may have been due to suffocation rather than chemical intoxication. Rainbow trout and fathead minnow are also potentially subject to suffocation in turbid environments. However, increased mucous production from the gills, which would exacerbate this effect, was not observed in this study.

In summary, the three basic plant designs (lagoon, aerated lagoon, mechanical-biological) were successful in reducing toxicity, regardless of time of year. Effluent from the eight plants was usually non-acutely toxic to the four test species, and induced in fish only minor disorders associated with the skin and gill lamella. Although influent was toxic to at least one species in 75% of the plants, there was no mortality at the other plants. The most sensitive species, *Anodonta grandis*, may have suffocated from high suspended solid loads, rather than have died from chemically induced intoxication.

Acknowledgements

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This study was initiated at the request of Municipal Branch (Standards and Approvals Division, Alberta Environment). Municipal Branch staff assisted in the development of the study design, made initial contact regarding the study with plant managers, arranged for chemical analysis of wastewater samples, and reviewed the final report. Dr. F. Skinner, Environmental Technology Division (Alberta Environmental Centre), provided useful advice on sample manipulation and ammonia removal from samples. Analysis of heavy metals in all samples was conducted by Water Analysis Branch (Alberta Environmental Centre).

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FISH TISSUE MONITORING AND BENTHIC INVERTEBRATE COMMUNITY ANALYSIS AT THE ALBERTA SPECIAL WASTE TREATMENT FACILITY, SWAN HILLS, ALBERTA.

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ABSTRACT

The 1991 fish tissue chemistry and benthic invertebrate monitoring programs at the Alberta Special Waste Treatment Facility are presented and methods for ensuring collection of ecologically meaningful data emphasized.

Levels of some organic and inorganic parameters in brook trout and white sucker tissue were found to be slightly above background levels. The possible sources of these compounds will be discussed. A discussion of the choice of appropriate detection limits and analytical techniques will be included, as these are of great importance in the Treatment Centre monitoring program, since many parameters are being measured near the detection limit.

Substantial changes in benthic invertebrate population abundance, community structure and trophic function occurred between years. Instantaneous measurements of water depth, velocity and substrate texture were the primary factors used to assess this variability. These parameters were not influenced by the facility and therefore the benthic program was not directly monitoring the effects of facility operations. It was recommended that the magnitude and fate of chemical inputs to the aquatic system be established before attempting to discern the influence they may have on community structure and function.

INTRODUCTION

Benthic invertebrate community analysis and fish tissue monitoring is conducted annually at the Alberta Special Waste Treatment Centre as part of a comprehensive environmental monitoring program. This monitoring serves as an early indication or potential effects of facility operation on the surrounding aquatic ecosystem.

Sampling sites were established in 1985, prior to facility start-up, and subsequent assessments use these data as a baseline for comparison. Sampling and analytical methodology are specified in a protocol document to ensure program continuity.

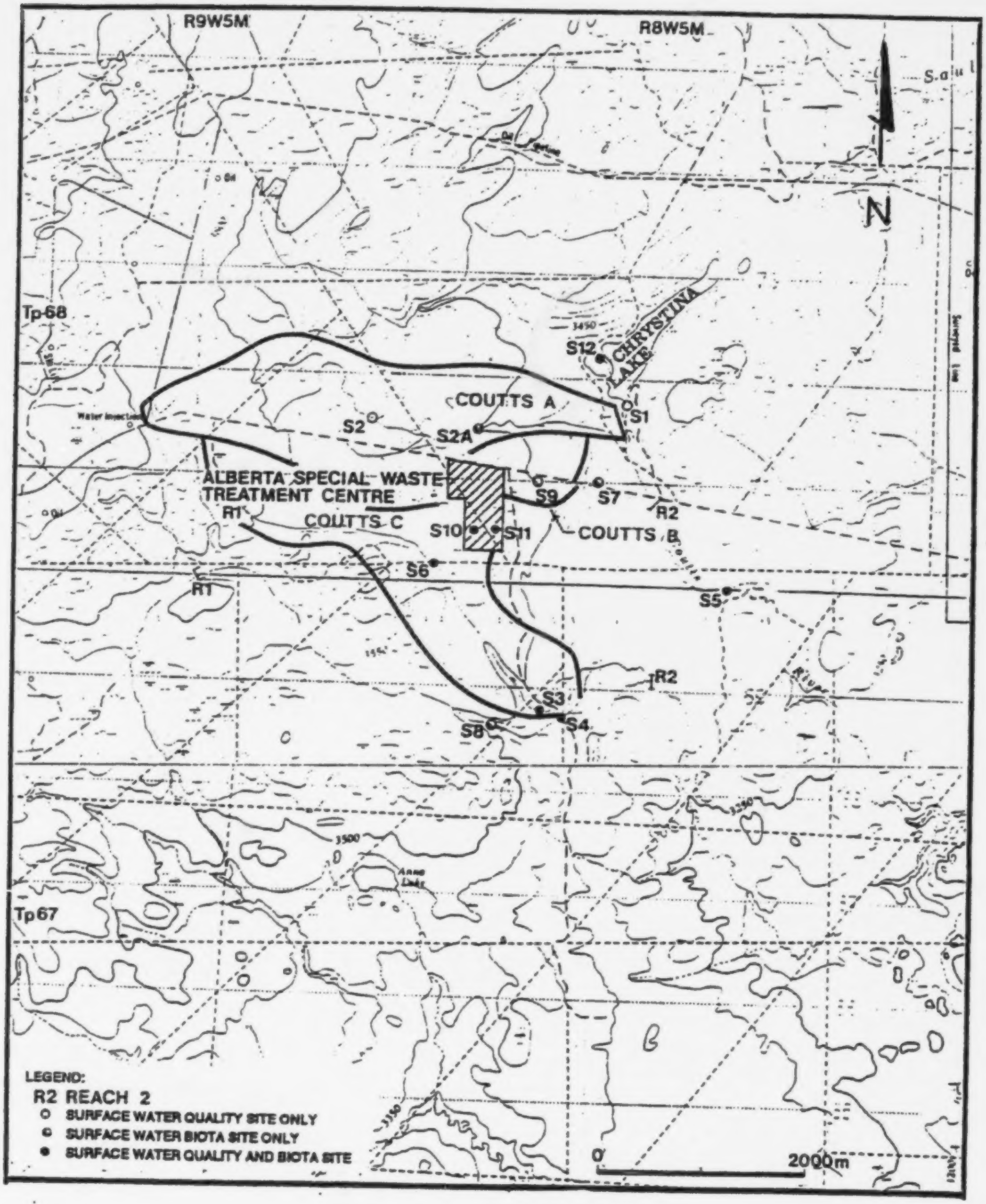
The following are results from the 1991 benthic and fish tissue assessments.

METHODS

Sample Collection

Benthic invertebrates were sampled at ten previously established sites in the Coutts River, it's tributaries, Chrystina Lake and on-site drainage ditches (Figure 1) between September 24th and October 2nd, 1991.

Figure 1. Surface Water Monitoring Location Plan


R9W5M
R8W5M
N
Tp 68
Tp 67
CHRYSTINA LAKE
3450
S12
COUTTS A
S1
S2
S2A
Water Injection
ALBERTA SPECIAL WASTE TREATMENT CENTRE
S9
S7
R2
R1
COUTTS C
S10
S11
COUTTS B
S6
R1
S5
R2
S3
S4
S8
3500
Anne Lake
3350
3150
LEGEND:
R2 REACH 2
○ SURFACE WATER QUALITY SITE ONLY
◐ SURFACE WATER BIOTA SITE ONLY
● SURFACE WATER QUALITY AND BIOTA SITE
0
2000m

Five benthic samples were collected from each lotic (moving water) site using a Neill-Hess sampler with a 250 μm mesh size and area of 0.0892 m^2. The lentic (standing water) benthos of Chrystina Lake (S12) was sampled with an Ekman dredge that enclosed an area of 0.0232 m^2.

The collected material was concentrated in the field by wet sieving and frozen with dry-ice. Sediment particle size distribution, water depth and current velocity were assessed with each benthic sample. All loose bed material was removed from the sampler, wet sieved and each size class weighed with a spring scale. Water depth and benthic current velocity (2 cm) measurements were taken immediately adjacent to each benthic sample with a Marsh-M'Birney current meter.

Large benthic samples were sub-sampled in the laboratory according to the procedures specified by Wrona et al., (1982). Invertebrates were picked from detritus under a dissecting microscope, separated according to taxonomic group, and preserved in denatured ethanol. Taxa were assigned to functional feeding groups according to the classification modified from Merritt and Cummins (1984).

Data Analysis

The assignment of sampling sites to specific habitat types was retained for the purposes of comparisons between sampling years. Riffle habitat had been previously designated as Reach 1 and included Sites 2A, S3, S4, S6, S7, and S8. Pool or run habitat, Reach 2, included only site S5 on the Coutts River. The on-site drainage ditches and Chrystina Lake were considered separately from this grouping.

The mean percentage by weight of sediment size classes, mean water depth and benthic current velocity was calculated for each site and compared to baseline data.

The number of taxa, standing crop and Shannon-Weaver species diversity were determined for each sample, means with 95% confidence limits were calculated for each site and plotted with baseline data (Figures 2,3, and 4).

Detrended Correspondence Analysis (DCA)(Hill 1979) was used to assess similarities and differences in invertebrate abundance between sites and years. This technique is similar to Reciprocal Averaging Ordination (RA) but the second axis is not systematically related to the first, making ecological trends easier to recognize. Secondly, axis units in DCA represent standardized changes in species composition; axis length increases with greater differences in species composition (Culp and Davies, 1983).

It should be noted that interpretation of ordination output is not as objective as has been suggested. The degree of separation and clustering of community data is related to the degree of data similarity; however, the delineation of clusters is subjective. As the degree of differences between sample sets changes so does the ability to distinguish clusters. It should also be emphasized that the causal mechanisms for clustering of data in an ordination (and community similarities) can only be inferred. Ordinations in themselves do not identify the factor(s) that are associated with the clustering.

Figure 2. Mean Number of Taxa with 95% Confidence Limits for all Sites and all Monitoring Years

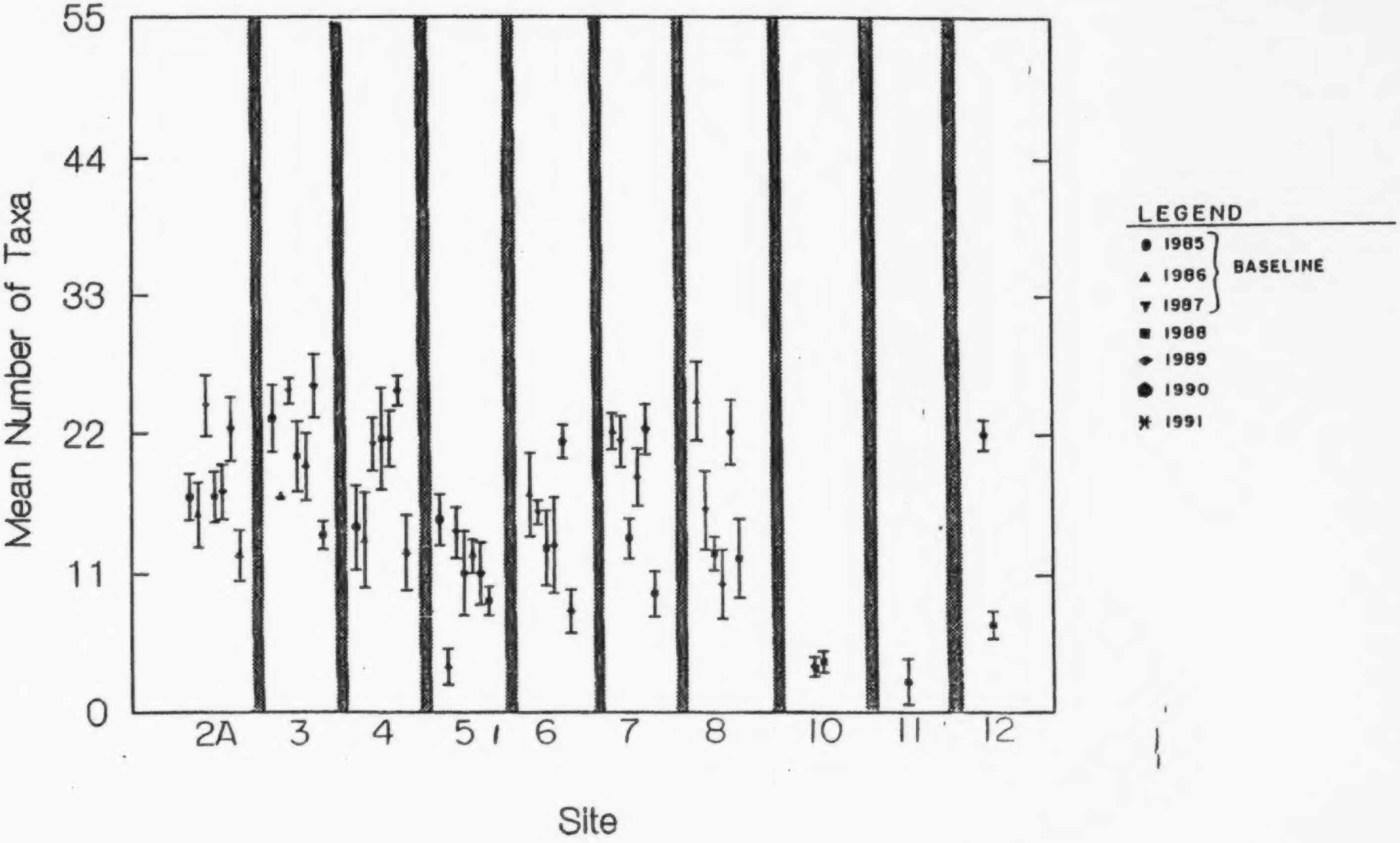


Figure 3. Mean Standing Crop with 95% Confidence Limits for all Sites and all Monitoring Years

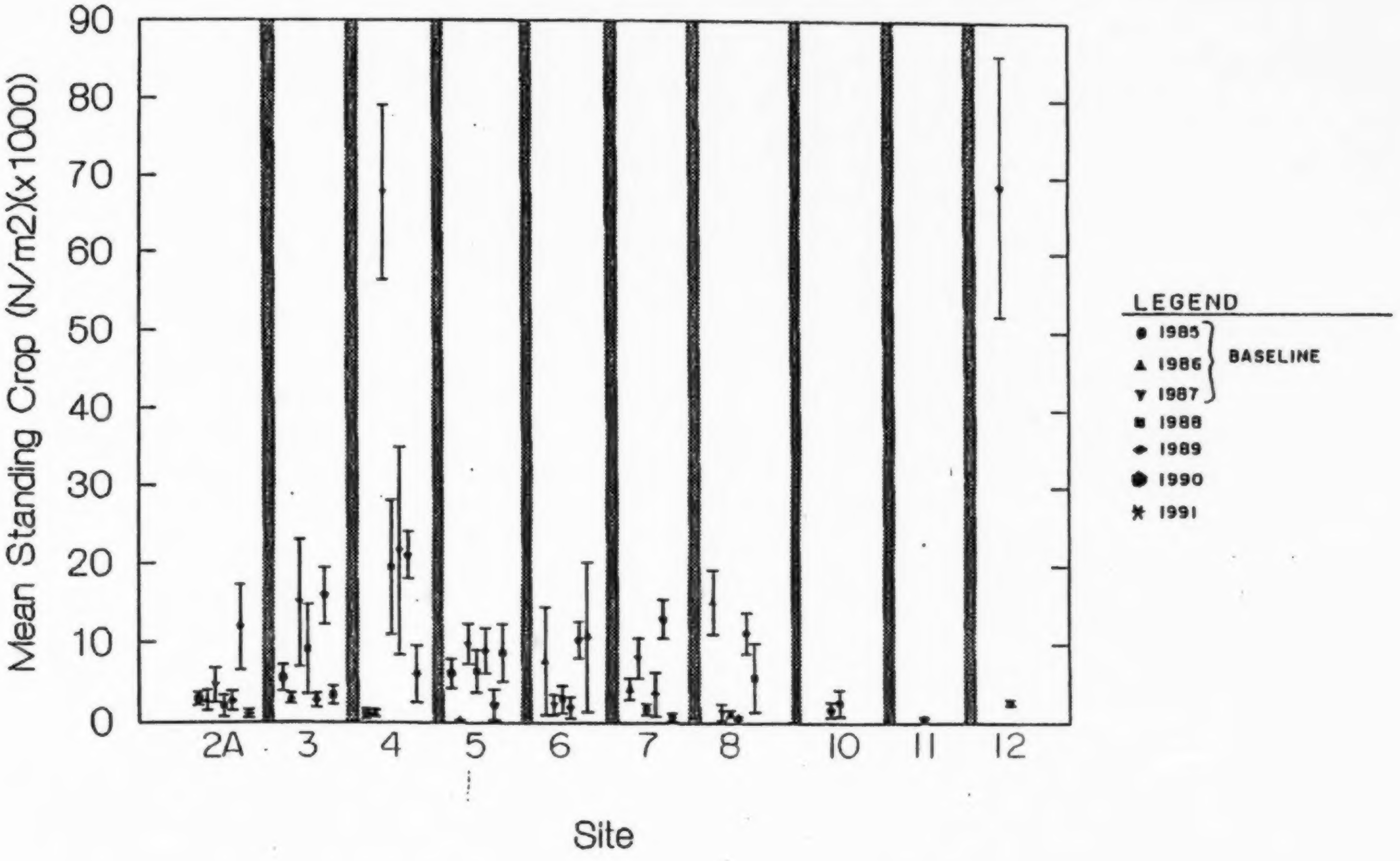
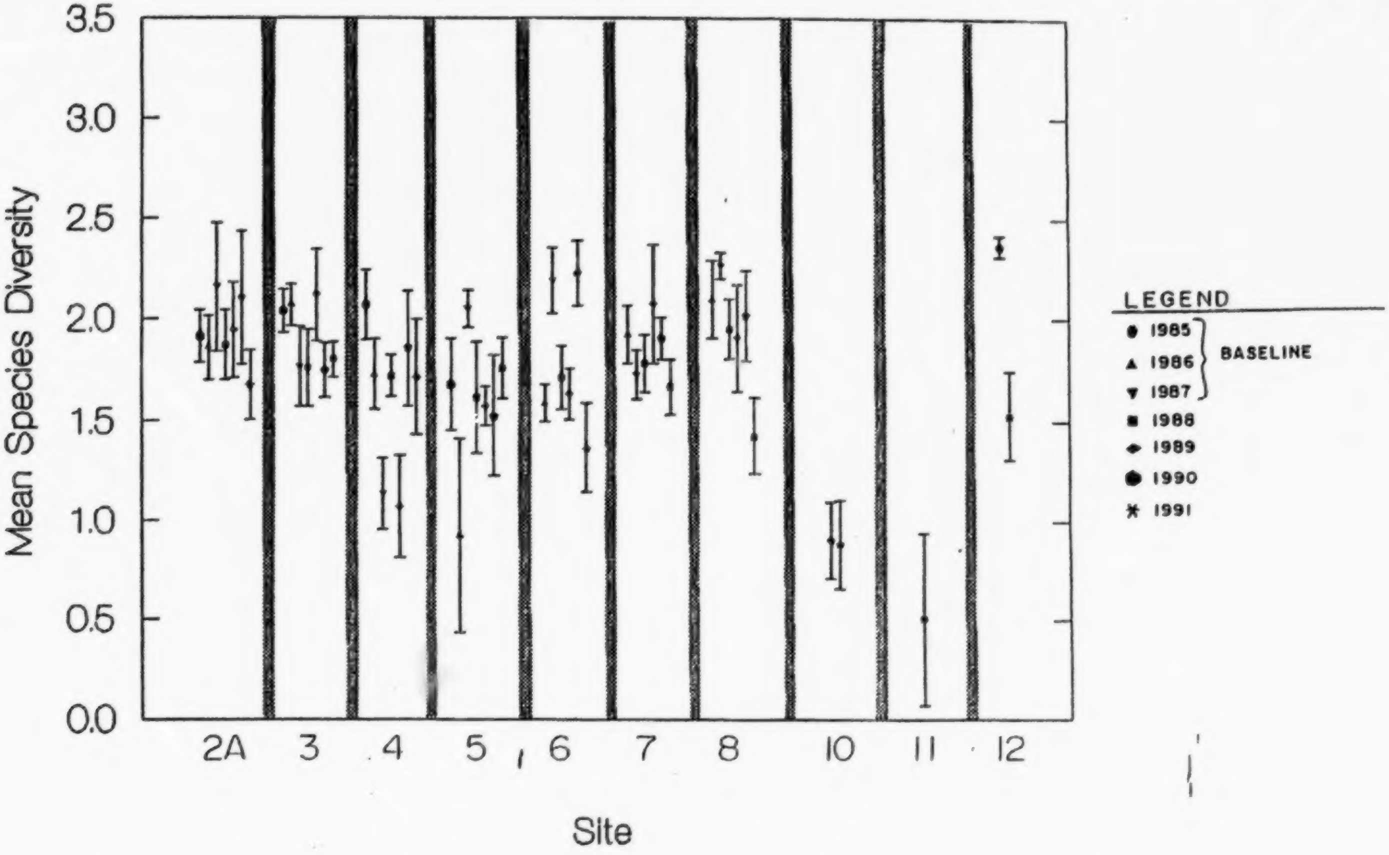


Figure 4. Mean Shannon-Weaver Diversity with 95% Confidence Limits for all Sites and all Monitoring Years



A trophic analysis of the 1991 population data was conducted by determining the percent composition of trophic classes. The number of aquatic invertebrates of each trophic class was determined for each sample and pooled means for each site compared to baseline data.

A number of new taxonomic units (tribes) were appended to the benthic invertebrate data base to simplify the classification of *Chironomidea* (midges) into trophic groups. The trophic function of *Chironomidea* can be defined by tribe (Merritt and Cummins 1984) thus reducing the need to identify these organisms to the species level. A total of 203 taxonomic units have now been defined to classify benthic invertebrates from the Coutts River, it's tributaries and Chrystina Lake.

RESULTS AND DISCUSSION

Due to site conditions some lotic sites were sampled with the Ekman dredge rather than the Neill-Hess sampler. After being placed on dry ice, benthic samples were generally frozen solid within one hour of sampling.

Physical Habitat

The stream bed material of benthic sampling sites (Table 1) was similar to baseline line however, water depth and flow velocities (Table 2) were considerably less then previous reports.

Coarse pebbles and cobbles were most prevalent in Reach 1 and finer materials prevalent in Reach 2 and on-site ditches, S10 and S11. The sediment in Chrystina Lake was slightly finer than previously reported, organic clay with only a few fine pebbles rather then the sand, clay organics and gravels sampled in 1990. Apparently, a more homogenous substrate was sampled in 1991.

Mean water depth and benthic current velocities were lower previous reports for all sites except S5 where water depth was within the background range. Flow velocities were unmeasurable at two sites due to insufficient water depth.

The benthic monitoring program was established so that all Reach 1 sampling sites were located in similar riffle habitats. In 1991, benthic current velocity, depth and sediment data suggest that there was considerable differences in flow regime and bed material composition between sites within Reach 1. Some discrepancies may have resulted from difficulty locating the appropriate study reaches due to inadequate marking of sites. Additionally, under low flow conditions similarities between sites diminished. In some sites (S4) beaver activity and similar factors have affected the hydraulic control of the study reaches altering the habitat conditions.

Basic Ecological Parameters

The total abundance of benthic invertebrates, mean number of taxa per sample (Figure 2) and standing crop (Figure 3) were considerably lower than in previous years while

Table 1. **Mean substrate size distribution (percentage by weight) for each benthic sampling site, baseline (1986-1987) and monitoring year 1991.**

Site	Year	No. Samples	Cobbles & Pebbles (>16 mm)	Fine-Med. Pebbles (16-4 mm)	V.Fine Pebbles (4-2 mm)	V.Coarse Sand (2-1 mm)
S2A	1985		96.5	3.2	0.3	<0.1
	1986		96.4	3.4	0.2	<0.1
	1987		97.6	2.4	0.1	-
	1991	5	99.6	0.4	0.0	0.0
S3	1985		94.9	4.9	0.6	0.1
	1986		92.7	6.3	0.8	0.1
	1987		91.7	7.3	0.8	0.2
	1991	5	92.4	5.5	1.2	0.9
S4	1985		98.8	1.1	0.1	-
	1986		98.5	1.4	0.1	<0.1
	1987		99.5	0.4	<0.1	-
	1991	5	99.2	0.8	0.0	0.0
S5	Visual Assessment: Organic silt					
S6	1986		99.2	0.7	0.1	-
	1987		99.4	0.5	0.1	<0.1
	1991	5	0.0	0.0	0.0	0.0
S7	1986		97.9	2.0	0.1	<0.1
	1987		96.5	2.9	0.4	0.1
	1991	5	74.8	1.4	0.3	23.5
S8	1986		93.8	5.6	0.3	0.2
	1987		96.6	3.0	0.3	0.1
	1991	5	99.5	0.3	0.0	0.2
S9	Visual assessment: Exposed bedrock, cobbles to clay					
S10	Visual assessment: Silty clay					
S11	Visual assessment: Sandy silt with some pebbles					
S12	Visual assessment: Organic clay with some fine pebbles					

Table 2. Mean water velocity and depth with 95% confidence limits for bethic sampling sites, baseline (1985-1987) and monitoring year 1991.

Site	Year	No. Samples	Mean Water Velocity (m/s)			Mean Water Depth (m)		
S2A	1985		0.54	+/-	0.07	0.19	+/-	0.02
	1986		0.37	+/-	0.08	0.16	+/-	0.02
	1987		0.23	+/-	0.03	0.1	+/-	0.02
	1991	-	NMF			0.03	+/-	0.01
S3	1985		0.43	+/-	0.04	0.15	+/-	0.02
	1986		0.37	+/-	0.14	0.09	+/-	0.01
	1987		0.25	+/-	0.08	0.09	+/-	0.02
	1991	-	NMF			0.04	+/-	0.01
S4	1985		0.41	+/-	0.10	0.20	+/-	0.02
	1986		0.41	+/-	0.15	0.16	+/-	0.02
	1987		0.26	+/-	0.04	0.13	+/-	0.03
	1991	15	<0.01	+/-	0.00	0.10	+/-	0.03
S5	1985		0.23	+/-	0.18	0.20	+/-	0.01
	1986		0.06	+/-	0.01	0.96	+/-	0.05
	1987		NMF			NMF		
	1991	15	0.02	+/-	0.00	0.61	+/-	0.08
S6	1986		0.13	+/-	0.05	0.24	+/-	0.03
	1987		0.06	+/-	0.02	0.18	+/-	0.03
	1991	15	0.02	+/-	0.01	0.11	+/-	0.02
S7	1986		0.38	+/-	0.10	0.2	+/-	0.02
	1987		0.30	+/-	0.06	0.15	+/-	0.02
	1991	15	0.01	+/-	0.03	0.07	+/-	0.01
S8	1986		0.49	+/-	0.08	0.24	+/-	0.02
	1987		0.24	+/-	0.06	0.1	+/-	0.01
	1991	15	0.02	+/-	0.01	0.04	+/-	0.01
S9	1991		NMF					
S10	1991		NMF					
S11	1991		DRY					
S12	1991		Not Measured					

NMF - Non-Measureable Flow due to insufficient depth.
Velocity = <0.01 - nondetectable flow

the Shannon-Weaver diversity index (Figure 4) was lower at some sites but not others. While there was a substantial decline in invertebrate abundance, the relative abundance of at least the numerically dominant taxa was reduced only at some sites. There was no pattern in species diversity that was consistent with prevailing winds or distance from the facility.

The 1991 invertebrate population and diversity data for stream sites suggest that the factor associated with the decline in invertebrate abundance and standing crop was not strongly selective for certain taxa and exerted a broad influence on the benthic community as a whole.

While some differences in sampling efficiency and taxonomy identification may be expected between researchers, this alone cannot explain the depleted benthic communities found. The Neill-Hess sampler utilizes water flow to capture invertebrates and therefore is less efficient under low flow conditions. Every effort was made to agitate the benthos and capture invertebrates but the reduced efficiency of the sampler, relative to previous years may have contributed to the apparent decrease in invertebrate abundance in shallow streams.

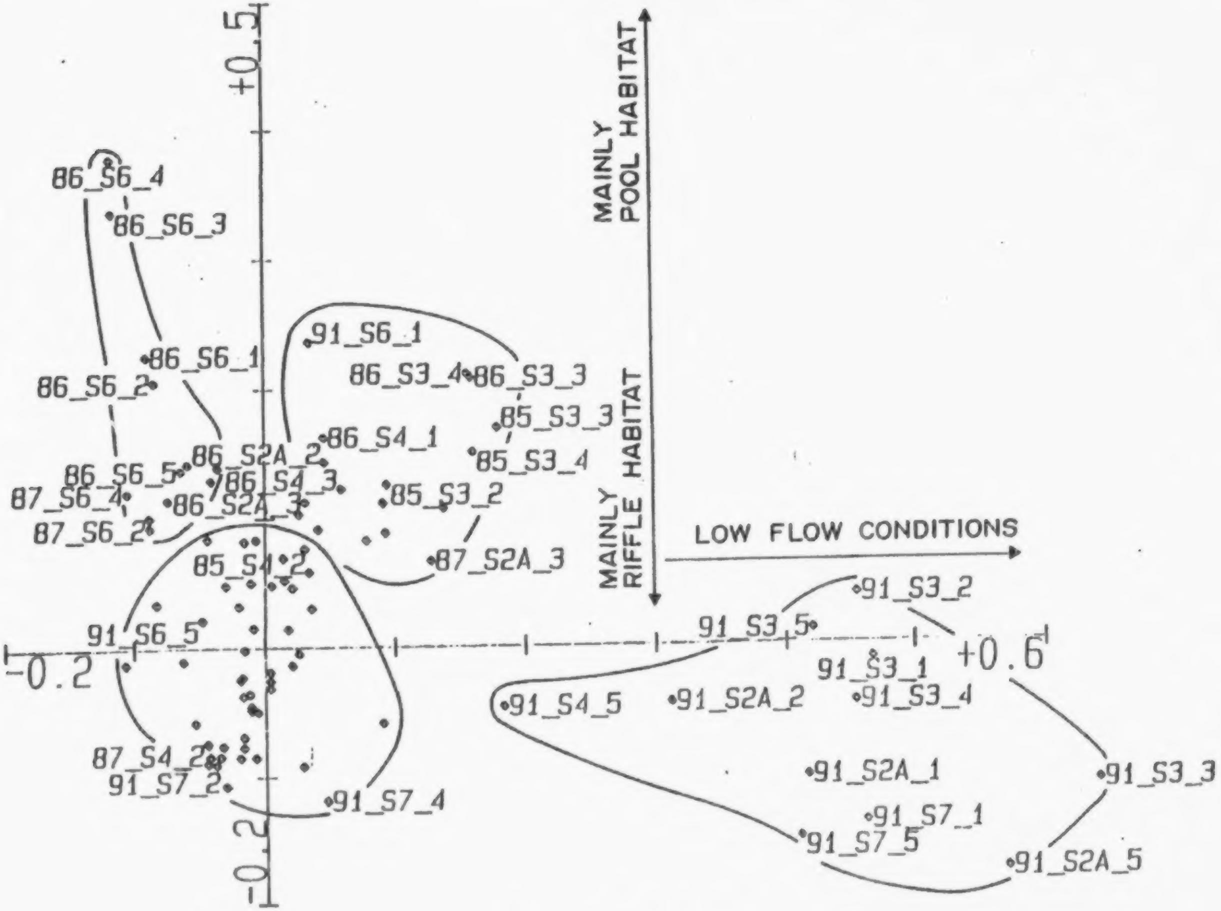
Community Analysis

The basic habitat gradients of fast moving riffle and moderate velocity run habitats were evident in the Reach 1, ordination (Figure 5) where vertical position and separation were maintained relative to previous years. The 1991 data were frequently clustered to one side of the vertical axis which suggested that, while the distinctions between communities still existed, some factor had influenced lotic community structure in a variety of habitat types.

The particularly low flow recorded during sampling could be associated with these changes in invertebrate community structure. Unless streams are morphologically identical, benthic habitats and the associated communities are expected to respond differently to equivalent changes in discharge. Due to changes in temperature, aeration, and shear stress the benthic community in shallow, high gradient streams may be more susceptible to change by a decrease in discharge that a deeper, low gradient stream.

The horizontal position along the X-axis may thus represent the degree of benthic community change that has occurred in each site under low flow conditions. In Reach 1, the samples clustered on the right, along the X-axis were, almost without exception, shallow riffles where current velocity could not be measured. These are habitats expected to be greatly affected by change in discharge and hence their extreme position along the X-axis. They are still riffle habitats, however, as indicated by the physical habitat data and suggested by their position on the Y-axis relative to previous years. Deeper riffles such as S4, S7 and S8 were apparently not as affected by the different flow regime and are clustered with previous years' data. The benthic community data from S6, a relatively incised, moderately flowing stream, was only slightly skewed and the site only marginally affected by low flow.

Figure 5. DCA Ordination of Invertebrate Abundance Reach 1, Baseline (1985-87) and 1991, Species Counts Less than 4 Removed



Ordinations of Reach 2, S5 (Figure 6) and Chrystina Lake, S12 (Figure 7), revealed similar trends where 1991 data maintained approximately the same vertical position but were skewed to the right. These temporal differences may be related to differences in the habitat sampled. Bed material in benthic samples from Chrystina Lake during 1991 was consistently fine textured substrate rather then the mix of fine and coarse sediment previously reported. Apparently, benthic samples were previously taken in more heterogenous habitats where greater species diversity would be expected.

Trophic Analysis

The trophic structure of all benthic communities sampled in 1991 was substantially changed relative to baseline data (Table 3). While there was a general decrease in the abundance of all trophic classes, the abundance and number of taxa of detritivores in particular was reduced and the proportion of carnivores and omnivores increased relative to baseline data.

The disproportional decrease in detritivores suggested that the factors responsible for reducing invertebrate abundance did not preferentially affect particular species, but rather a particular trophic group; those that utilize detritus as a food source. It is unlikely that the input of leaves to streams may have changed in one year but the retention of this material may have been lower than previous years. Stream discharge was particularly high in the spring of 1991 and may have flushed more detritus out of the sample reaches than usual. Detritivore populations may thus have been reduced by a decrease in the amount of available food.

CONCLUSIONS AND RECOMMENDATIONS

Flow velocity, water depth and, to a lesser extent, sediment data suggest that there were considerable habitat differences between sites that were initially selected as being similar riffle habitats. The flows found in 1991 were the lowest recorded and during low flow conditions, differences in benthic habitat became more apparent. It should be emphasized that the measurements taken in this program are instantaneous and the benthic community is shaped by the immediate short-term history of these parameters. Beaver dams, dead fall and other changes in stream morphology have also altered the physical habitat of the study reaches since collection of baseline data.

A particularly diminished benthic community was found in 1991 compared to baseline data. The total abundance of benthic invertebrates, mean number of taxa and standing crop found was considerably less than all previous reports. The Shannon-Weaver diversity index was lower only at some sites and there was no apparent pattern in species diversity consistent with prevailing winds or distance from the facility.

The distinction between riffle and run/pool benthic invertebrate communities continued to be evident in the vertical separation of data in the benthic community ordinations. 1991 Data were frequently skewed to one side of previous data yet vertical position and separation were maintained relative to the same sites in previous years. This

Figure 6. DCA Ordination of Invertebrate Abundance, Reach 2, S5, Baseline 1985-1987 and 1991

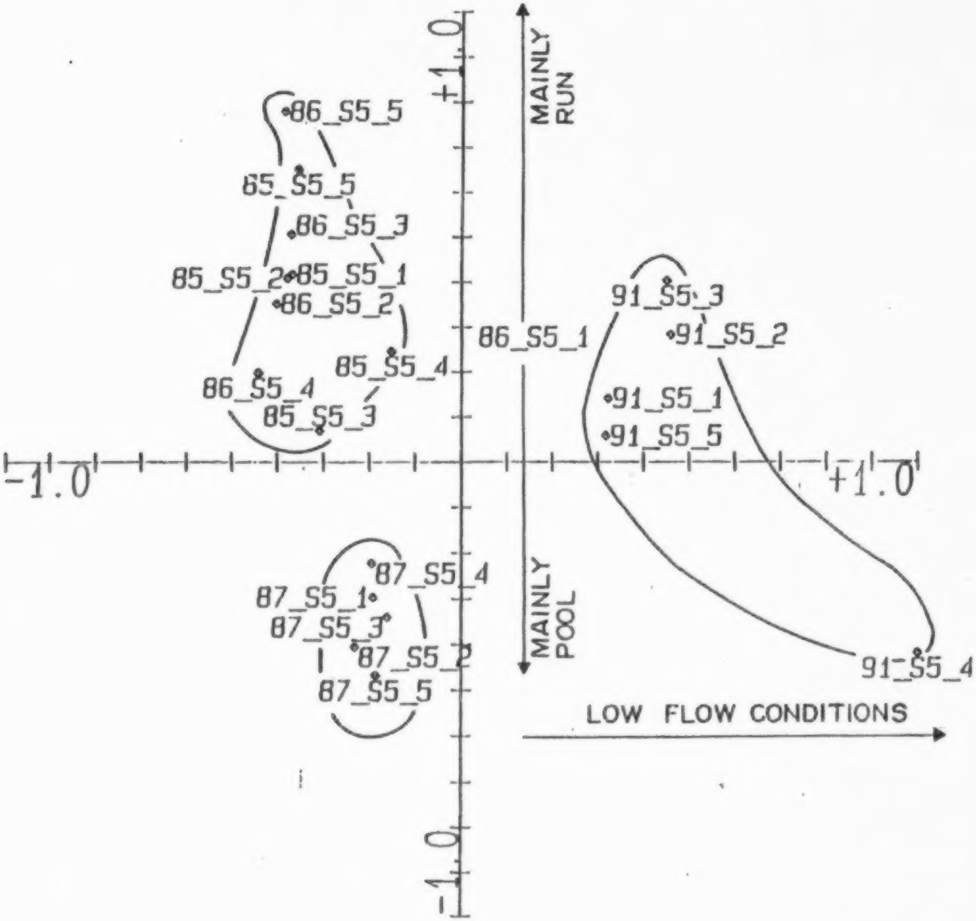


Figure 7. DCA Ordination of Invertebrate Abundance for S12, Chrystina Lake, 1990 and 1991 Species Counts Less than 3 Removed

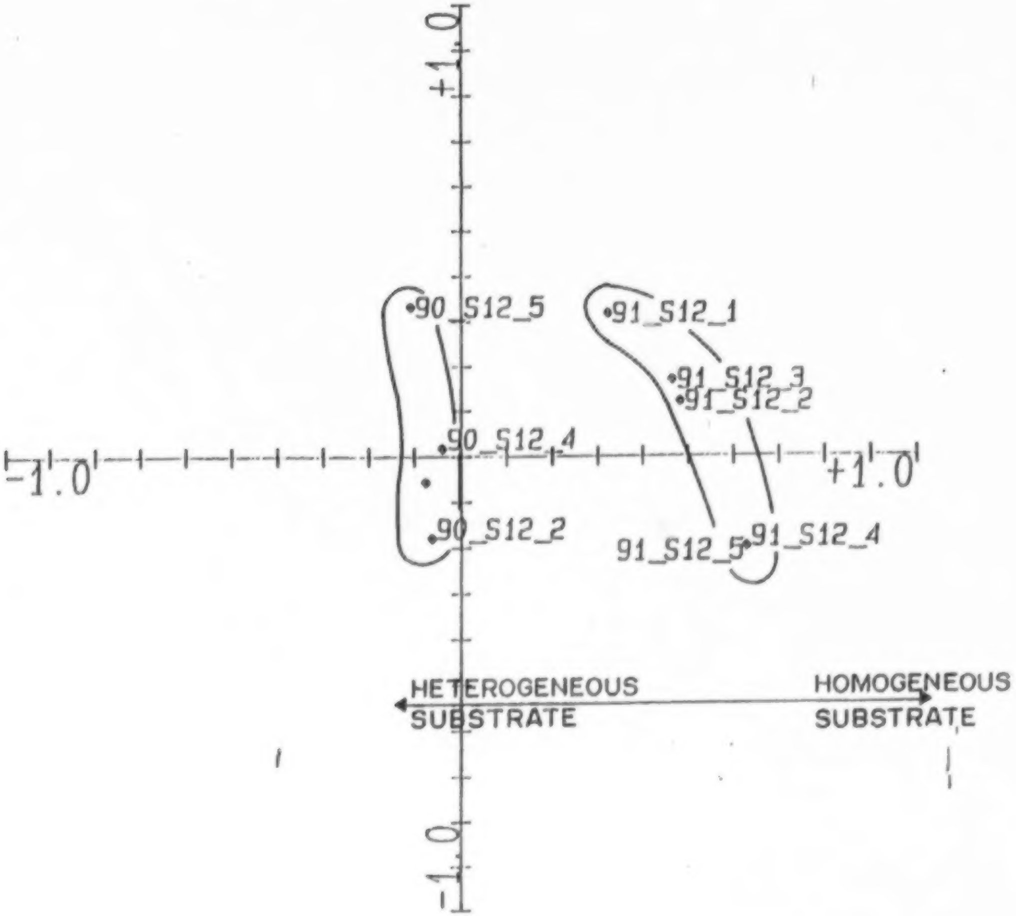


Table 3. Percent composition of benthic invertebrate trophic classes for each sampling site (pooled), baseline (1985-1987) and monitoring year 1991.

Site	Year	Trophic Class (percent)							
		Carnivore	Carnivore/ Detritivore	Detritivore	Detritivore/ Herbivore	Herbivore	Herbivore Carnivore	Omnivore	Other
S2A	1985	3.6	1.0	89.6	4.2	0.1	0.0	1.5	
	1986	2.3	0.9	79.9	8.7	0.0	0.0	8.2	
	1987	4.7	2.3	71.3	18.5	0.8	0.0	2.4	
	1991	20.8	1.9	36.4	16.5	0.0	0.0	24.2	0.2
S3	1985	2.8	2.0	73.9	19.8	0.0	0.0	1.5	
	1986	2.2	1.7	62.8	32.7	0.0	0.0	0.5	
	1987	2.6	0.4	83.9	12.9	<0.1	0.0	0.1	
	1991	17.4	1.2	36.5	29.2	0.0	0.0	15.6	0.0
S4	1985	14.3	0.6	78.3	3.5	0.0	0.0	3.3	
	1986	10.5	1.7	82.2	3.4	0.0	0.0	2.1	
	1987	3.1	0.8	95.1	0.4	0.0	0.0	0.7	
	1991	47.1	1.0	1.0	1.7	0.0	0.0	49.2	0.0
S5	1985	4.6	0.0	49.1	0.1	0.0	0.0	46.2	
	1986	5.8	0.0	34.8	0.0	0.0	0.0	59.4	
	1987	14.2	0.0	73.0	0.1	0.0	0.0	12.6	
	1991	56.5	4.4	3.8	0.0	0.1	0.0	35.1	0.0
S6	1986	1.9	0.5	52.6	0.2	0.1	0.0	44.6	
	1987	1.4	0.1	77.9	11.1	0.0	0.0	9.5	
	1991	44.7	1.6	2.3	0.3	0.0	0.0	51.1	0.0
S7	1986	5.4	1.9	84.2	7.3	0.3	0.0	0.9	
	1987	4.9	0.6	86.2	8.3	0.0	0.0	0.1	
	1991	43.9	1.2	9.1	13.0	0.0	0.0	32.7	0.0
S8	1986	3.0	0.9	80.3	4.6	1.2	0.0	10.0	
	1987	6.9	0.8	79.7	5.9	0.2	0.0	6.5	
	1991	37.1	1.8	1.5	2.1	0.0	0.0	57.6	0.0
S10	1990	61.1	2.1	35.2	1.6	0.0	0.0	0.0	
	1991	72.9	13.0	0.7	0.0	0.0	0.0	13.4	0.0
S11	1991	52.3	0.0	4.6	0.0	0.0	0.0	43.2	0.0
S12	1990	6.6	1.7	90.3	0.1	0.5	0.0	0.8	
	1991	42.9	0.0	1.0	0.3	0.0	0.0	55.9	0.0

suggested that, while the distinctions between communities still existed, some factor had modified benthic community structure in a variety of habitat types.

The trophic structure of all benthic communities sampled in 1991 was substantially changed relative to baseline data. While there was a general decrease in abundance of all trophic classes, the abundance and number of taxa of detritivores in particular was reduced and the proportion of carnivores and omnivores increased.

Substantial changes in the physical environment and reduction in suitable habitat or food resources are some common factors that can be associated with these types of community changes. Specifically, extreme changes in stream discharge, particularly high flows in the spring of 1991 and very low flows in the fall may have resulted in an overall decrease in invertebrate abundance and local extinction of previously rare species. High flows can have a direct effect on benthic communities, flush decaying leaves and temporarily reduce available food resources for detritivores. Low flows can reduce the availability of suitable habitat as well as sampler efficiency.

The benthic invertebrate monitoring program detected changes in basic ecological parameters and community structure. Without more extensive monitoring of stream stage and discharge, the causal mechanisms for these changes can only be inferred and it is unlikely that the benthic program, as specified in the existing monitoring protocol, could discern the effects of facility operation from hydrological and hydraulic influences. The physical habitat and community structure is apparently more variable than originally anticipated.

While it may appear to be more cost effective to suspend the existing program, it is preferable that benthic data become an integral part of the water and sediment monitoring programs. If statistically significant differences in water or sediment quality are found, the benthic program will be used to evaluate the ecological significance of these changes. While the published literature can, to some extent, be used for this evaluation, one of the best site-specific indications of potential effects is that realized by organisms in close association with both water and sediment - benthic invertebrates. Benthic communities are influenced by a variety of physical and biological factors which must be rigorously monitored before the effects of non-point source discharge, such as that from the Treatment Centre, can be assessed. In order to improve the existing program it is therefore recommended that the continuous monitoring of stream stage and discharge be implemented during the spring and summer months.

FISH TISSUE ANALYSIS METHODS

Sample Collection

Between September 24th and October 2nd 1991, 36 Eastern brook trout (*Salvelinus fontinalis* (Mitchill) and 14 white suckers (*Catostomus commersoni*) were captured from Chrystina Lake (Figure 1) using gill nets. Fork length and weight were measured and age classes tentatively identified in the field. Five fish from each of three apparent age classes of brook trout were sacrificed for tissue analysis as well as several individuals that did not fit this classification. Five suckers of similar size were also sacrificed. Each fish was filleted on a piece of plastic wrap to cover the working surface using new scalpel blades and gloves for each fish. One filet of each fish was wrapped in organic free aluminium foil for analysis of specified organics and the other placed in plastic wrap for inorganic analysis. Filets were placed in zip lock bags, and frozen on dry ice for analysis of parameters specified in the monitoring protocol document.

Laboratory Analysis

Trout age was confirmed by scale and otolith analysis. Five fish of each age class were selected for preparation of composite samples by the laboratory. Inductively coupled plasma (ICP) was used for inorganic analysis and EPA protocol 8270 was used for semi-volatile organics.

1991 trout samples were compared to the 1985 to 1987 data, prior to facility start-up while white sucker tissue was compared to 1990 results as there was no baseline data for this species. There was insufficient replication for statistical comparisons.

Published reports of fish contaminants are occasionally based on wet weight concentrations while the 1991 data were on a dry weight basis. The water content of fish tissue samples was not assessed and therefor a correction factor of 0.223 was used to estimate wet weight concentrations (77.7% water content, Watt and Merril 1963).

RESULTS AND DISCUSSION

Only three age classes of brook trout (1+, 2+ and 3+) were captured from Chrystina Lake in 1991. Similar reports from local anglers and Alberta Fish and Wildlife support this apparent decline in the abundance of older trout.

Age determinations by otolith and scale examinations were subject to considerable subjective interpretation as annuli were indistinct. In stocked populations of brook trout, such as those present in Chrystina Lake, it has been recommended that otoliths of known age be retained at the time of stocking in order to provide a reference for future age determinations (Mackay et al., 1990). In a reference manual recommended by Alberta Fish and Wildlife, Cox and Ralston (1990) present all fish contaminant data according to fish length rather than age in order to minimize problems associated with fish ageing.

There are currently no standards in Canada for assessing chemical composition of fish tissue in relation to fish mortality or fecundity (reproductive success). The Guidelines for Freshwater Aquatic Life (CCREM 1991) and numerous toxicological studies discuss water quality in relation to the health of aquatic biota but do not relate tissue concentrations to biota health or mortality. Environment Canada is working with Ontario's Ministry of the Environment to establish criteria for "residue" or "body burden" analyses but these standards are not yet available for public use.

Inorganic Parameters

Numerous inorganic parameters exceeded the 1985 to 1987 brook trout baseline levels particularly for 2+ and 3+ fish (Table 4). Six parameters exceeded baseline for 1+ trout, 21 parameters exceeded baseline in 2+ trout and 15 parameters exceeded baseline in 3+ trout. 13 inorganic parameters exceeded 1990 concentrations in White suckers (Table 5).

Arsenic and beryllium concentrations in brook trout were low but elevated above background levels for all age classes for the first time in 1991. Iron and sodium were also elevated in all age classes but are common, naturally occurring elements expected in fish tissue.

Table 4. Inorganic parameter concentrations in Brook Trout tissue from Chrystina Lake. Baseline (1985-1987) and monitoring year 1991.

AGE CLASS	1+				2+			3+			
PARAMETER ug/g	1985	1986	1987	1991	1985	1986	1991	1985	1986	1987	1991
Aluminum	14	106	35.2	13	9.5	95.9	14	2.8	100	9.8	13
Antimony	<0.02	<0.03	0.82	0.05	<0.02	<0.03	0.14 +	<0.02	<0.03	1.18	0.37
Arsenic	<0.02	0.09	0.41	0.71 +	<0.02	0.09	0.40 +	<0.02	0.11	0.82	0.97 *
Barium	0.41	0.13	1.29	0.9	0.23	0.12	0.7 +	0.05	<0.03	1.11	1.1
Beryllium	<0.02	<0.03	<0.1	0.2 *	<0.02	<0.03	0.3 *	<0.02	<0.03	<0.1	0.2 *
Bismuth	<0.02	<6	0.4	<2.5	<0.02	<6	<2.5	<0.02	<6	<0.3	<2.5
Boron	<0.2	<0.3	<1.0	<1	2.3	<0.3	<1	0.3	0.58	<1.0	<1
Cadmium	<0.04	<0.06	0.7	0.4	<0.04	<0.06	<0.1	<0.04	<0.06	0.7	<0.1
Calcium	3030	439	1480	1130	2130	368	890	720	249	1750	1330
Chromium	<0.04	0.22	2.8	1.9	<0.04	0.21	1.7 +	<0.04	0.35	1.4	2.4 +
Cobalt	<0.10	<0.16	3.2	<0.1	<0.10	<0.16	1.0 +	<0.10	<0.16	2.2	0.5
Copper	0.22	0.40	6.7	2.1	0.42	0.60	2.3 +	0.81	0.30	11.3	2.4
Iron	5.64	23.2	37.6	42 +	5.89	29.2	41 +	4.1	27.7	42.1	44 *
Lead	<1.0	<0.16	7.0	1	<1.0	<0.16	<1	<1.0	<0.16	1.5	2.4 +
Lithium	<1.0	<1.6	<0.5	<0.5	<1.0	<1.6	<0.5	<1.0	<1.6	<0.5	<0.5
Magnesium	338	256	1290	1230	324	222	1160 +	286	246	1050	1150 +
Manganese	0.80	0.190	1.77	1.6	0.58	0.270	1.2 +	0.10	0.120	1.48	1.6 +
Mercury	0.11	0.08	0.08	0.312 *	0.17	0.09	0.286 +	0.30	0.10	0.119	0.352 *
Molybdenum	<0.2	<0.3	3.4	0.7	<0.2	<0.3	0.4 +	<0.2	<0.3	2.0	0.6
Nickel	<0.2	<0.3	5.3	<1	<0.2	<0.3	2.2 +	0.4	<0.3	5.5	<1
Phosphorus	4220	2230	12800	10900	3490	2000	10800 +	2600	2120	10100	10500 +
Potassium	4400	3670	18200	18700 *	4220	3440	19900 *	4180	3740	14150	18200 +
Selenium	0.06	0.03	0.14	0.04	0.1	0.04	0.04	0.06	0.02	0.44	0.03
Silicon	4.0	<1.6	13.3	9	2.0	<1.6	7.6 +	1.0	<1.6	2.6	11 +
Silver	-	-	<0.1	<0.1	-	-	0.2	-	-	<0.1	<0.1
Sodium	571	400	1650	1810 *	520	453	2000 *	498	489	1290	2040 *
Strontium	4.04	0.19	2.5	1.7	2.5	0.15	1.3	0.39	<0.03	2.5	2
Thallium	-	-	<0.3	<0.1	-	-	<0.1	-	-	<0.3	<0.1
Thorium	<2	<3.0	<5.0	<5	<2	<3.0	<5	<2	<3.0	<5.0	<5
Titanium	3.96	1.46	<1.0	2.7	2.2	1.35	2.8 +	2.2	1.38	<1.0	2.7 +
Uranium	<6	<10	<1.0	9 *	<6	<10	<5	<6	<10	<1.0	15 *
Vanadium	<0.04	<0.06	2.1	0.5	<0.04	<0.06	0.7 +	<0.04	<0.06	1.3	0.8
Zinc	14.1	6.83	41.8	34.8	9.03	7.26	34.2 +	7.1	4.31	30.2	32.3 +
Zirconium	<0.10	<0.16	0.7	<0.5	<0.10	<0.16	<0.5	<0.10	<0.16	<0.5	<0.5

Non detected - Represented by less than (<) the detection limit.

+ - exceeds all background concentrations for that age class.

* - exceeds all background concentrations in all age classes.

Table 5. Inorganic parameter concentrations in White Sucker tissue from Chrystina Lake, monitoring years 1990 and 1991.

PARAMETER ug/g	1990	1991
Aluminum	13	16
Antimony	0.01	0.08
Arsenic	<0.01	0.40
Barium	21	2.0
Beryllium	0.1	0.2
Bismuth	2.3	<2.5
Boron	22	<1
Cadmium	<0.1	<0.1
Calcium	15800	850
Chromium	3.6	2.0
Cobalt	0.3	<0.1
Copper	2.8	2.0
Iron	44	44
Lead	<1	1
Lithium	<0.1	<0.5
Magnesium	1380	1250
Manganese	9.8	1.5
Mercury	0.177	0.471
Molybdenum	<0.1	<0.1
Nickel	0.4	<1
Phosphorous	16500	10500
Potassium	16000	20400
Selenium	<0.01	0.04
Silicon	25	7
Silver	<1	0.4
Sodium	2060	2300
Strontium	23	<1
Thallium	0.1	<0.1
Thorium	2.27	<5
Titanium	0.5	2.5
Uranium	<5	7
Vanadium	0.1	1.0
Zinc	42.4	30.9
Zirconium	<0.005	<0.5

Arsenic bioconcentration is generally low and a biological half-life of 7 d has been reported (Sorenson 1976 in CCREM 1991). There is no evidence of arsenic biomagnification (Demayo et al. 1979 in CCREM 1991). Bioconcentration of beryllium is considerably higher (BCF 19 to 100) and there is no evidence of biomagnification (CCREM 1991).

Mercury was elevated above background levels for all age classes in 1991 as well as in 1990. The concentration reported for 3+ trout was only slightly higher than in 1985, prior facility start-up. White suckers had the highest mercury content reported to date, 0.471 µg/g, consistent with their benthic feeding habit.

Total mercury levels in fish from Canadian inland, natural waters are typically below 0.5 µg/g while elevated mercury levels of between 2.73 to 10.5 µg/g have been reported (CCREM 1991). Compared to these elevated levels, the mercury concentrations in fish tissue from Chrystina are relatively low. The level of Chrystina Lake has been recently raised and soil flooding may lead to elevated mercury levels in associated aquatic ecosystem due to bacterial alkylation of soil mercury.

Chromium, titanium, and zinc were elevated above background concentrations in the two oldest age classes of trout; however, these results are within the background range of the 1+ age class. Magnesium, manganese, phosphorus, potassium, and silica were similarly elevated but these are expected in fish tissue at the concentrations reported.

The lead concentration in 3+ trout (2.4 µg/g dry weight) is greater than background levels for that age class and may be high compared to other reports. CCREM (1991) reports that lead concentrations in fish muscle tissue from areas of known local lead contamination were 1.78 µg/g while lesser contaminated areas had fish tissue concentrations of less than 0.5 µg/g. If the 1991 data are converted to wet weight (0.5 µg/g ,Table 6), lead concentration was within expected ranges. The background (1987) lead concentration in 1+ brook trout tissue, 7 µg/g (1.6 µg/g wet weight) appeared unexpectedly high, and suggested methodological difficulties.

Uranium concentrations in 1+ and 3+ trout (9 and 15 µg/g respectively) exceeded background levels and were at least one order of magnitude greater than other reports, even when corrected for water content (2 and 3 µg/g, Table 6). In waters receiving uranium mine tailings, uranium concentrations in fish muscle tissue of less 0.100 µg/g (Lockhart et al. in press) and between 0.190 and 0.290 µg/g (CCREM 1991) have been reported. The particularly high uranium concentrations found in this program appear to be due to analytical difficulties resulting from unacceptably high detection limits.

Organic Parameters

In 1991, PCB concentrations in fish tissue apparently decreased to less than the practical quantification limit 0.004 µg/g (Table 7). Prior to 1989 the concentration of PCBs in trout tissue was less than the detection limit of 0.5 µg/g. Low level PCB analysis of 1989 fish extracts and 1990 fish tissue indicated PCB levels between 0.026 and 0.043 µg/g, and 0.010 and 0.055 µg/g, respectively.

Table 6. Wet weight equivalent of inorganic parameters in Brook Trout tissue from Chrystina Lake, monitoring year 1991.

AGE CLASS	1+		2+		3+	
PARAMETER	CONCENTRATION Dry Weight ug/g	CONCENTRATION Wet Weight ug/g	CONCENTRATION Dry Weight ug/g	CONCENTRATION Wet Weight ug/g	CONCENTRATION Dry Weight ug/g	CONCENTRATION Wet Weight ug/g
Aluminum	13	3	14	3	13	3
Antimony	0.05	0.01	0.14	0.03	0.37	0.08
Arsenic	0.71	0.16	0.40	0.09	0.97	0.22
Barium	0.9	0.2	0.7	0.2	1.1	0.2
Beryllium	0.2	0.0	0.3	0.1	0.2	0.0
Bismuth	2.5	0.6	2.5	0.6	2.5	0.6
Boron	1	0	1	0	1	0
Cadmium	0.4	0.1	0.1	0.0	0.1	0.0
Calcium	1130	252	890	198	1330	297
Chromium	1.9	0.4	1.7	0.4	2.4	0.5
Cobalt	0.1	0.0	1.0	0.2	0.5	0.1
Copper	2.1	0.5	2.3	0.5	2.4	0.5
Iron	42	9	41	9	44	10
Lead	1.0	0.2	1.0	0.2	2.4	0.5
Lithium	0.5	0.1	0.5	0.1	0.5	0.1
Magnesium	1230	274	1160	259	1150	256
Manganese	1.6	0.4	1.2	0.3	1.6	0.4
Mercury	0.312	0.070	0.286	0.064	0.352	0.078
Molybdenum	0.7	0.2	0.4	0.0	0.6	0.1
Nickel	1	0	2.2	0.5	1	0
Phosphorus	10900	2431	10800	2408	10500	2342
Potassium	18700	4170	19900	4438	18200	4059
Selenium	0.04	0.01	0.04	0.01	0.03	0.01
Silicon	9.0	2.0	7.6	0.0	11.0	2.5
Silver	0.1	0.0	0.2	0.0	0.1	0.0
Sodium	1810	404	2000	446	2040	455
Strontium	1.7	0.4	1.3	0.3	2.0	0.4
Thallium	0.1	0.0	0.1	0.0	0.1	0.0
Thorium	5	1	5	1	5	1
Titanium	2.7	0.6	2.8	0.6	2.7	0.6
Uranium	9	2	5	1	15	3
Vanadium	0.5	0.1	0.7	0.2	0.8	0.2
Zinc	34.8	7.8	34.2	7.6	32.3	7.2
Zirconium	0.5	0.1	0.5	0.1	0.5	0.1

Wet Weight = Conc. (Dry Weight) X 0.223

Depuration, the reduction in tissue concentration, of PCBs has been shown in a number of organisms (CCREM 1991). Most marine organisms were shown to eliminate 50% of the lower chlorinated PCBs in one to several weeks and higher chlorinated PCBs in one to two months. It is possible that 1990 fish tissue PCB concentrations were metabolized to current levels. Using the highest tissue concentration reported in 1990, 0.055 µg/g, and the longest half-life cited, two months, PCB levels could be depurated to below the current detection limit within 8 months assuming no further assimilation of PCBs by fish after the 1990 sampling program. Sediment release rates of PCBs are slow however, and elimination of PCBs from the food chain at that rapid rate is unlikely. Therefore, the apparent reduction in fish tissue PCB levels seen in 1991 cannot be explained by depuration alone.

As PCBs are lipophilic, the decrease in PCB tissue content may be due to differences in fat (lipid) content in fish tissue samples between years. The proportion of body fat may have been lower in 1991 than in previous years. Differences in fat content may have also resulted from the preparation of tissue samples in that primarily muscle tissue was collected in 1991, and fat, bones and blood vessels were excluded where possible. Sampling protocol has now been modified to ensure consistency and collection of tissue samples in a manner similar to that which anglers might consume.

Phthalates were detected in several fish tissue samples in 1991 but are extremely common and expected whenever a sample is in contact with plastic.

Trace concentrations of polycyclic aromatic hydrocarbons (PAHs) were found for the first time in 1+, 3+ aged brook trout and white suckers. While some PAHs may be produced by forest fires, microbial activity and plants, a significant portion of PAHs are a result of the incomplete combustion of organic materials such as fuels and refuse. Atmospheric deposition of PAHs is believed to be a common route of entry to aquatic systems although land-based entry and direct discharge to water may also be significant. Materials containing creosote, crude oil and hydrocarbon products are all potential sources of PAHs in aquatic systems. (CCREM 1991)

PAHs are bio-concentrated in aquatic organisms relative to dissolved concentrations and rates vary with molecular weight and configuration. In general, trout rapidly uptake both low and high molecular weight PAHs but metabolism and depuration are also rapid. The biological half-life of two and three-ring PAHs in rainbow trout (*Oncorhynchus mykiss*) was shown to be 6 to 9 days (CCREM 1991).

A chlorinated phenol not previously detected, 2,3,4,6 tetrachlorophenol, was found in trace concentrations in 3+ brook trout. Pentachlorophenol and 4-nitrophenol, found in fish tissue in 1985, were not detected in this program. Chlorinated phenols are released to the aquatic environment by a variety of sources including pulp and paper operations, wood treatment plants, and agricultural products. Until 1983, 2,3,4,6 tetrachlorophenol was manufactured in Alberta but production has stopped (CCREM 1991). Bio-concentration of chlorinated phenols increases with the degree of chlorination and may reach 1000 for higher chlorinated forms. Uptake from the water is rapid but depuration rates are also rapid with an expected biological half-life in fish of less than 10 days for the higher chlorinated phenols (CCREM 1991).

Table 7. Concentration of organic parameters in fish tissue from Chrystina Lake monitoring year 1991.

	TROUT AGE CLASS			WHITE SUCKER
AGE CLASS	1+	2+	3+	
PARAMETER (ug/g)				
PCB's	<0.004	<0.004	<0.004	<0.004
Ethers	<0.066	<0.066	<0.066	<0.066
Phthalates		<0.330		
Bis(2-ethylhexyl)	1.107	<0.330	<0.330	<0.330
Butylbenzyl	<0.033	<0.033	<0.033	<0.330 X >0.033
Diethyl	<0.033	<0.033	<0.033	<0.330 X >0.033
Dimethyl	<0.330 X >0.033	<0.033	<0.033	2.79
Di-n-butyl	<0.033	<0.033	<0.330 X >0.033	<0.330 X >0.033
Nitrosamines	<0.132	<0.132	<0.132	<0.132
Base/Neutrals	<0.330	<0.330	<0.330	<0.330
Polyaromatic		<0.150		
Benzo (a) anthracene	<0.330 X >0.033	<0.033	<0.033	<0.033
Fluoranthene	<0.330 X >0.033	<0.033	<0.033	<0.033
2-methylnaphthalene	<0.033	<0.033	<0.330 X >0.033	<0.033
Phenanthrene	<0.033	<0.033	<0.033	<0.330 X >0.033
Chlorinated	<0.120	<C.120	<0.120	<0.120
Phenols	<0.165	<0.165		<0.165
2,3,4,6 tetrachlorophenol			<0.660 X >0.066	

Non detected - Represented by less than (<) the detection limit. When classes of compounds are cited the highest detection limit of the specified parameters are used.

Trace concentrations - Represented as less than (<) the quanitation limit but greater than (>) detection limit.

CONCLUSIONS AND RECOMMENDATIONS

Large brook trout were not captured in 1991 and the analysis of tissue was conducted on fish apparently three years old and younger. Ageing of stocked trout is subject to

considerable subjective interpretation and it is recommended that future evaluations be conducted on the basis of fork length and weight rather than age class.

The concentration of several inorganic parameters in brook trout and white sucker tissue were elevated above background levels. Arsenic and beryllium were slightly elevated for all age classes and should continue to be monitored closely. Mercury concentrations, while elevated, are relatively low compared to other Canadian data and may be due to phenomena not associated with the waste treatment facility. Chromium, titanium, and zinc were elevated only in the two oldest age classes but were within the range of background levels when age was not considered.

The lead concentration in 3+ brook trout was greater than background levels; however some of the background data were far greater than expected from other reports. Uranium levels were above background in the youngest and oldest age classes and an order of magnitude greater than the other reports. The detection limit for these analyses was not sufficiently low enough to detect the level of contamination expected in fish tissue. It is therefore recommended that all specified detection limits be re-assessed and analytical techniques utilized to ensure that ecologically meaningful data are being collected.

PCBs were not detected in any fish tissue samples - an apparent reduction from 1990 levels. Decreases due to depuration of PCBs is a possibility but methodically differences between sampling programs may have also contributed to the decrease. It is recommended that fish tissue sampling protocol be modified to standardize or at least assess fat content in fish samples.

Trace concentrations of polyaromatic hydrocarbons and chlorinated phenol were found for the first time in all but the 2+ age class of brook trout. PAH's are relatively common hydrocarbons with a variety of natural and anthropomorphic sources.

In conclusion, the concentration of a number of organic and inorganic parameters have been slightly elevated relative to baseline condition. Temporal trends will continue to be closely monitored. Improvements to the fish monitoring program have been made to ensure that this program serves as an early indicator of potential environmental concerns.

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CORRELATION BETWEEN PCP AND 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN EQUIVALENTS IN OIL, SOIL, AND GROUNDWATER AT WOOD PRESERVING SITES.

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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins and related furans (PCDDs/PCDFs) are common production impurities in commercial-grade pentachlorophenol (PCP). Data relating concentrations of PCP to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ) in oil, soil and groundwater have been collected at six historically-contaminated wood preserving sites in Alberta. Though the size of the data set is limited (n=25), several trends are apparent: the ratio TCDD-EQ/PCP ranges over 1 order-of-magnitude for oil, 3 for soil and 2 for groundwater, though the strongest TCDD-EQ/PCP correlation exists in oil (r^2=0.60) and soil (r^2=0.79); 2,3,7,8-TCDD is consistently identified as one of the PCDDs present, though its contribution to the aggregate TCDD-EQ of the mixture is minimal (<15%); and partitioning equilibrium theory approximates the ratios within a factor of three for a three-phase system (oil, soil, and groundwater). Analytical limitations and the presence of mixed phases are believed to limit the strength of the correlations more than variations in PCP manufacture. In any case, the uncertainty associated with the correlations presented herein is no greater than for many other parameters routinely used in quantitative risk assessment and site remediation design.

INTRODUCTION

Chemical wood preservation is used to protect timber from bacteria, fungi and insects which consume wood fibre as substrate, extending the useful life of wood products and thereby reducing the demand for replacement timber. Pentachlorophenol (PCP) has historically been the biocidal agent of preference for utility poles and fence posts. In PCP wood preservation, technical-grade PCP is blended with a diesel fuel, or No. 1/No. 2 fuel oil carrier to produce a 3 to 6% working solution. The means of applying this solution to timber with a specified end use is dictated by CSA Standard 080 -Wood Preservation (1989).
Formulations of technical-grade PCP predating the mid 1970s contained significant quantities of production impurities such as lower chlorophenols, chlorinated phenoxyphenols as well as polychlorinated dibenzo-*p*-dioxins (PCDDs) and associated furans (PCDFs) (Nilsson et al., 1978). The actual composition and amount of impurities is principally affected by the source material and manufacturing conditions (Crosby, 1981), though a typical profile has been established (Table 1). At present, PCP is a regulated chemical in North America, and the two active USA manufacturers are obligated by Federal law to maintain hexachlorodibenzo-*p*-dioxin levels below 2ppm (2E-06) (Mitchell, 1992).

Contamination at wood preserving sites is principally associated with chemical spills, drippage from freshly treated timber, and foremost, from the disposal of spent preservative solution. Released at or near the ground surface, PCP-hydrocarbon liquid progresses downward through the soil in response to gravity. As the oil infiltrates, a portion will be retained by the soil matrix against gravity due to interfacial tension manifested as wetting and capillary forces. The hydrocarbon so retained by the soil matrix against gravity is termed residual saturation. Consequently, with downward progression, the volume of free liquid is incrementally exhausted through conversion to residual saturation, and migration of the oil wetting front ceases. Successive oil-based contamination migrates undiminished through soil at residual hydrocarbon saturation, extending the wetting front eventually into the saturated zone, where three contaminated media (phases) exist: oil, soil and groundwater.

An illustrative representation of the complex interaction of the media in this groundwater system is illustrated in Figure 1. The idealized relationship assumed for partitioning computations is presented as Figure 2. Partitioning relations predict the ratios of the contaminant in the various environmental and polluting media at equilibrium, and are based on the premise that interphase chemical equilibrium is the lowest chemical potential attainable in a system, and as such, all systems will strive to attain this potential. The principal limitation of partitioning equilibria theory at these sites is the presence of mixed (versus distinct) phases. Rapid media flux relative to partitioning kinetics may also limit the accuracy of partitioning estimates at some sites.

Table 1. Typical Composition of Technical-Grade PCP (Hoffmann and Hrudey, 1990)

Compound Family	Component	Typical % in Technical Grade PCP
Chlorinated Phenols	Pentachlorophenol (PCP)	84.6 - 90.4
	Tetrachlorophenol	3.0 - 10.4
	Trichlorophenol	0.002 - 0.100
	Chlorinated phenoxy-phenols	6.2 - 7.0
Polychlorinated Dibenzo-*p*-dioxins	Octachloro-	0.0015 - 0.33
	Heptachloro-	0.0007 - 0.087
	Hexachloro-	0.0001 - 0.0038
	Pentachloro-	nd - 0.000008
	Tetrachloro-	nd - 0.000125
Polychlorinated Dibenzofurans	Octachloro-	nd - 0.0300
	Heptachloro-	0.00018 - 0.0400
	Hexachloro-	0.00034 - 0.0090
	Pentachloro-	nd - 0.0040
	Tetrachloro-	nd - 0.0125

Figure 1. Interaction Between Media in a Three-Phase (Oil, Soil, Water) System (Hoffman and Hrudey, 1990)

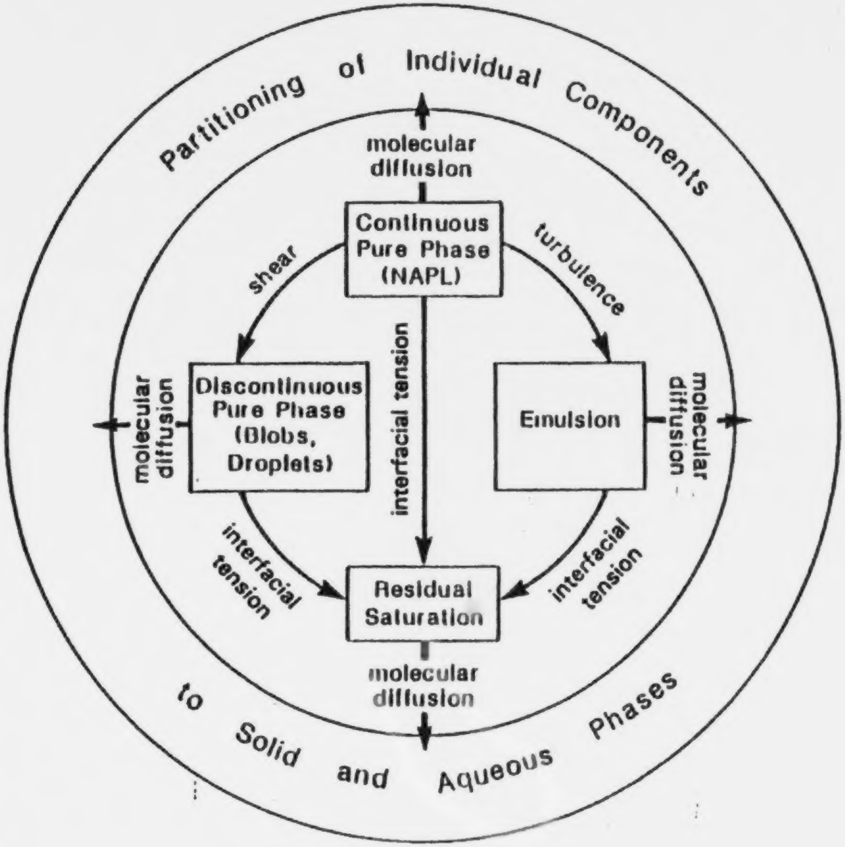
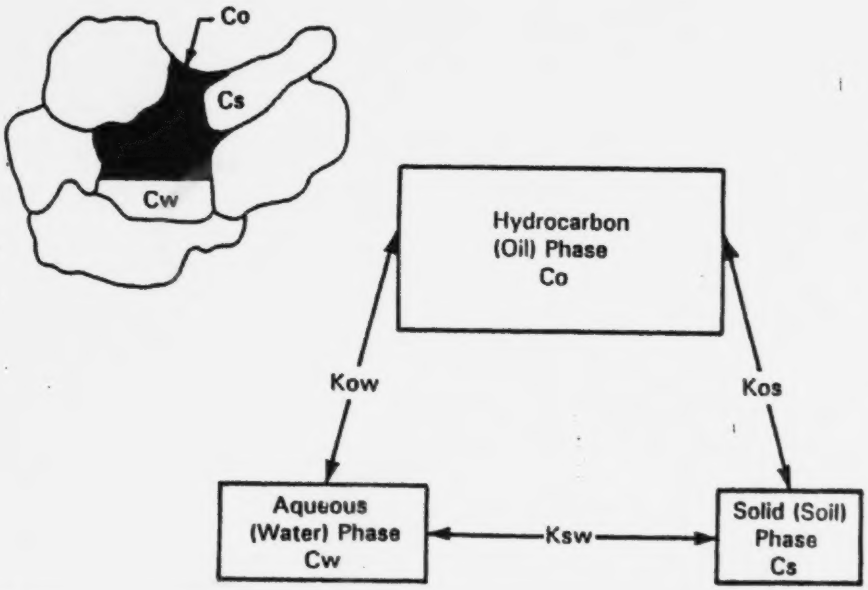

Partitioning of Individual Components
molecular diffusion
Continuous Pure Phase (NAPL)
shear
turbulence
interfacial tension
molecular diffusion
Discontinuous Pure Phase (Blobs, Droplets)
Emulsion
molecular diffusion
interfacial tension
interfacial tension
Residual Saturation
molecular diffusion
to Solid and Aqueous Phases

Figure 2. Partition Equibilria Relationships


Co
Cs
Cw
Hydrocarbon (Oil) Phase Co
Kow
Kos
Aqueous (Water) Phase Cw
Ksw
Solid (Soil) Phase Cs

- C_{oi} = Concentration of contaminant i in oil.
- C_{wi} = Concentration of contaminant i in water.
- C_{si} = Concentration of contaminant i in soil.
- $K_{owi} = C_{oi}/C_{wi}$
- $K_{osi} = C_{oi}/C_{si}$
- $K_{swi} = C_{si}/C_{wi}$

developed using linear regression. The least squares (best fit) line was constrained to pass through the origin, on the premise that PCP is the sole source of the PCDDs/PCDFs present. This hypothesis is consistent with data in that PCDDs/PCDFs have not been detected in the absence of PCP in any sample.

RESULTS AND DISCUSSION

Correlation Constants

The TCDD-EQ/PCP regressions for oil, soil, and groundwater are presented in Tables and Figures 3, 4, and 5, respectively.

Table 2. Data Set

Site	Period of PCP Use	Sample Reference	PCP Concentration*1 (Times 1E−06)	TCDD−EQ Concentration*2 (Times 1E−12)	R (TCDD−EQ/ PCP)	Sample Description*3	Data Usefulness
A	1958−1982	A1	16000	194560	1.2E−05	oil, soil, water (oil−saturated sediment from sludge pond)	used
		A2	900	28001	3.1E−05	soil, residual oil (hydrocarbon−stained soil)	used
		A3	300	6116	2.0E−05	soil	used
		A4	500	5138	1.0E−05	soil	used
B	1954−1962	B1	460	6780	1.5E−05	oil [DNAPL, LNAPL], groundwater (oil flowing into trench)	used
		B2	4000	120805	3.0E−05	oil [LNAPL]	used
		B3	3100	96019	3.1E−05	oil [DNAPL]	used
		B4	4300	12420	2.9E−06	oil [DNAPL]	used
		B5	4500	18110	4.0E−06	oil [DNAPL]	used
		B6	11	2270	2.1E−04	soil	used
		B7	79	6080	7.7E−05	soil	used
		B8	0.945	0.2192	2.3E−07	groundwater	used
C	1963−1987	C1	0.150	0.0021	1.4E−08	groundwater	used
		C2	1.20	0.04272	3.6E−08	groundwater	used
		C3	0.730	405.5	5.6E−04	oil [LNAPL] (subsampled from mixed LNAPL/groundwater sample)	disqualified*4
		C4	4200	8351	2.0E−06	oil [DNAPL] (no visibly entrained soil or groundwater)	used
		C5	3.2	4995	1.6E−03	oil [DNAPL] (subsampled from mixed DNAPL/groundwater/LNAPL sample)	disqualified*4
D	1947−1988	D1	39	124.0	3.2E−06	soil	used
		D2	883	25676	2.9E−05	oil [LNAPL], water (preserving oil in open tank)	used
		D3	1230	134.6	1.1E−07	soil, residual oil (hydrocarbon−stained soil)	used
		D4	14.3	43.5	3.0E−06	soil	used
E	1954−1987	E1	0.130	0.1144	8.8E−07	groundwater, oil [DNAPL, LNAPL]	used
		E2	0.020	0.1899	9.5E−06	groundwater	used
		E3	0.075	0.1194	1.6E−06	groundwater, dispersed oil phase (droplets)	used
		E4	0.450	24837	5.5E−02	oil [DNAPL] (subsampled from mixed DNAPL/groundwater/LNAPL sample)	disqualified*4
		E5	69.0	33870	4.9E−04	oil [LNAPL] (subsampled from mixed LNAPL/groundwater sample)	disqualified*4
F	1967−1970	F1	4800	55028	1.1E−05	soil, residual oil (hydrocarbon−stained soil)	used
		F2	1300	22802	1.8E−05	soil, residual oil (hydrocarbon−stained soil)	used
		F3	430	4293.4	1.0E−05	soil	used

Notes:

*1: exponent 1E−06 = mg PCP/kg dry soil; mg PCP/L water; ug PCP/g oil

*2: exponent 1E−12 = pg TCDD−EQ/g dry soil; ng TCDD−EQ/L water; pg TCDD−EQ/g oil

*3: constituent phases of sample are listed in order of contribution to sample volume. The sample is classified as either oil, soil, or groundwater for correlation assessment based on the dominant phase.

*4: PCP analysis conducted on composite−phase sample, whereas PCDD/PCDF analysis performed only on subsample of oil−phase, thereby increasing the concentration of TCDD−EQ relative to PCP.

Eight data from four sites were used to correlate TCDD-EQ and PCP in the hydrocarbon (oil) phase (Table and Figure 3). The ratios range over one order-of-magnitude (2.0E-06 to 3.1E-05). The correlation is statistically weak (r^2=0.60), though a goodness-of-fit test indicates the variables are correlated with a 5% probability this inference is incorrect. The slope of the least squares line is 1.2E-05.

Eleven data from four sites were used to relate TCDD-EQ and PCP in soil (Table and Figure 4). The ratios vary widely (1.1E-07 to 2.1E-04), though a moderately-strong correlation exists (r^2=0.79; goodness-of-fit test indicates variables are correlated with less than a 1% chance of error). The slope of the best fit line is 1.2E-05.

Six data from 3 sites were used to correlate TCDD-EQ and PCP in groundwater (Table and Figure 5). The ratios vary over two orders of magnitude (1.4E-08 to 9.5E-06) and the variables are statistically uncorrelated.

Based on the correlation coefficients (r^2), these regressions do not provide statistically rigorous relations for oil, soil or groundwater. I believe the presence of mixed phases, coupled with analytical limitations, contribute more to the order-of-magnitude variation in the results than variability in PCP manufacture, which would be expected to provide variation within one order-of-magnitude. Consequently, improving sampling practices to collect distinct phases is the obvious target to reduce data dispersion. However, by the very nature of the interactions between environmental and polluting media, only limited advances in this area may be possible.

On a more pragmatic level, these correlations are useful and do not contribute excessive uncertainty: generalized constants of 1E-05 for oil and soil, and 1E-07 for groundwater cannot but improve upon the status quo where PCDDs/PCDFs are either omitted from consideration or where their concentrations are estimated using assumed constants with no scientific basis. Furthermore, site remediation design and quantitative risk assessment routinely incorporate data with equal or greater uncertainty than is associated with the foregoing correlation constants. For example: groundwater modelling of pump-and-treat remediation is fraught with order-of-magnitude uncertainties for heterogeneous formations and where NAPLs are present (Mackay and Cherry, 1989); and dose-response relationships used in risk assessment are generally considered to provide several orders-of-magnitude uncertainty (Sielken, 1987).

Presence of 2,3,7,8-TCDD

Technical-grade PCP is generally considered not to contain 2,3,7,8-TCDD (World Health Organization, 1987; Mitchell, 1992), a belief which is emphatically propagated by PCP suppliers, who might otherwise face substance reclassification. However, considering the presence of pentachlorodibenzofuran is readily acknowledged, it does not take great faith to expect that trace amounts of a structurally similar compound, 2,3,7,8-TCDD, might also be present.

2,3,7,8-TCDD was detected in oil or soil at 3 of 6 sites. It was present in 6 of 29 samples, at concentrations as high as 23ng/g. However, concern over the presence of this compound is tempered in that it contributes relatively little to the aggregate TCDD-EQ of the mixture (<15%). A conventional risk assessment considering 2,3,7,8-TCDD in isolation would

Table 3. & Figure 3. Oil Data

Table 3: Oil Data

Site	Sample Reference	PCP Concentration	TCDD-EQ Concentration	R (TCDD-EQ/ PCP)	Sample Description*1
A	A1	1.60E-02	1.95E-07	1.2E-05	oil, soil, water (oil-saturated sediment from sludge pond)
B	B1	4.60E-04	6.78E-09	1.5E-05	oil [DNAPL, LNAPL], groundwater (oil flowing into trench)
	B2	4.00E-03	1.21E-07	3.0E-05	oil [LNAPL]
	B3	3.10E-03	9.60E-08	3.1E-05	oil [DNAPL]
	B4	4.30E-03	1.24E-08	2.9E-06	oil [DNAPL]
	B5	4.50E-03	1.81E-08	4.0E-06	oil [DNAPL]
C	C4	4.20E-03	8.35E-09	2.0E-06	oil [DNAPL] (no visibly entrained soil or groundwater)
D	D2	8.83E-04	2.57E-08	2.9E-05	oil [LNAPL], water (preserving oil in open tank)

Notes:

*1: constituent phases of sample are listed in order of contribution to sample volume. The sample is classified as either oil, soil, or groundwater for correlation assessment based on the dominant phase.

Regression Output:

Constant	0
r Squared	0.60
No. of Observation	8
Best Fit R	1.2E-05

Figure 3: Oil Data

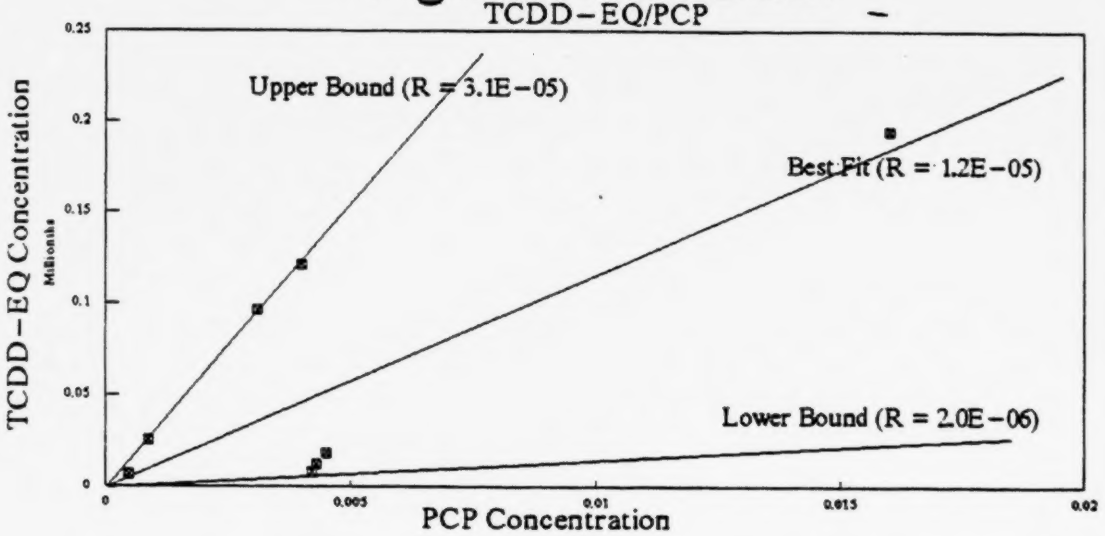


Table 4: Soil Data

Site	Sample Reference	PCP Concentration	TCDD-EQ Concentration	R (TCDD-EQ/ PCP)	Sample Description*1
A	A2	9.00E-04	2.80E-08	3.1E-05	soil, residual oil (hydrocarbon-stained soil)
	A3	3.00E-04	6.12E-09	2.0E-05	soil
	A4	5.00E-04	5.14E-09	1.0E-05	soil
B	B6	1.10E-05	2.27E-09	2.1E-04	soil
	B7	7.90E-05	6.08E-09	7.7E-05	soil
D	D1	3.90E-05	1.24E-10	3.2E-06	soil
	D3	1.23E-03	1.35E-10	1.1E-07	soil, residual oil (hydrocarbon-stained soil)
	D4	1.43E-05	4.35E-11	3.0E-06	soil
F	F1	4.80E-03	5.50E-08	1.1E-05	soil, residual oil (hydrocarbon-stained soil)
	F2	1.30E-03	2.28E-08	1.8E-05	soil, residual oil (hydrocarbon-stained soil)
	F3	4.30E-04	4.29E-09	1.0E-05	soil

Notes:

*1: constituent phases of sample are listed in order of contribution to sample volume. The sample is classified as either oil, soil, or groundwater for correlation assessment based on the dominant phase.

Regression Output:

Constant	0
r Squared	0.79
No. of Observation:	11
Best Fit R	**1.2E-05**

Figure 4: Soil Data

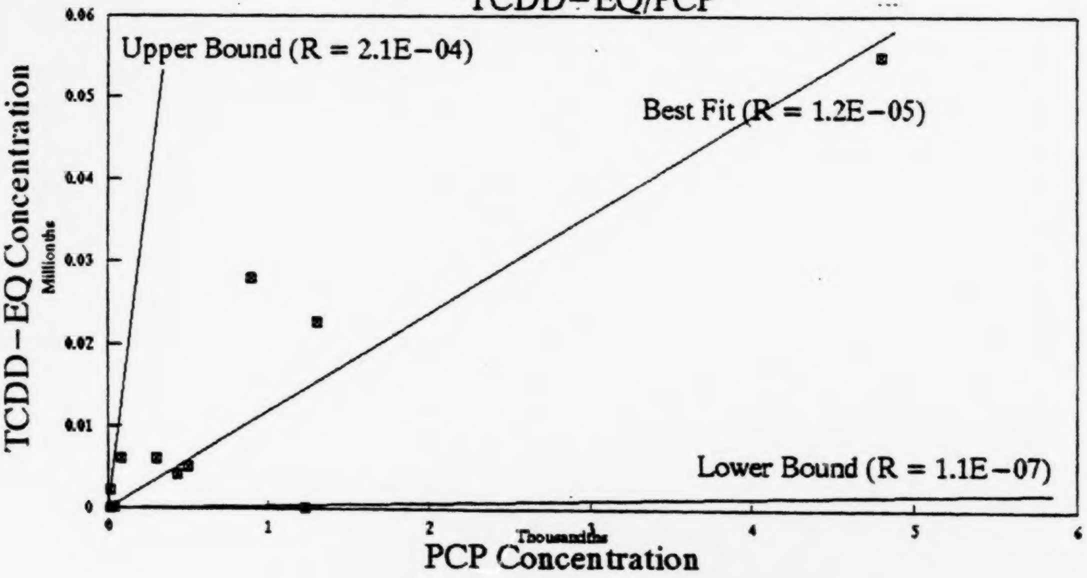


Table 5: Groundwater Data

Site	Sample Reference	PCP Concentration	TCDD-EQ Concentration	R (TCDD-EQ/ PCP)	Sample Description*1
B	B8	9.45E-07	2.19E-13	2.3E-07	groundwater
C	C1	1.50E-07	2.10E-15	1.4E-08	groundwater
	C2	1.20E-06	4.27E-14	3.6E-08	groundwater
E	E1	1.30E-07	1.14E-13	8.8E-07	groundwater, oil [DNAPL, LNAPL]
	E2	2.00E-08	1.90E-13	9.5E-06	groundwater
	E3	7.50E-08	1.19E-13	1.6E-06	groundwater, dispersed oil phase (droplets)

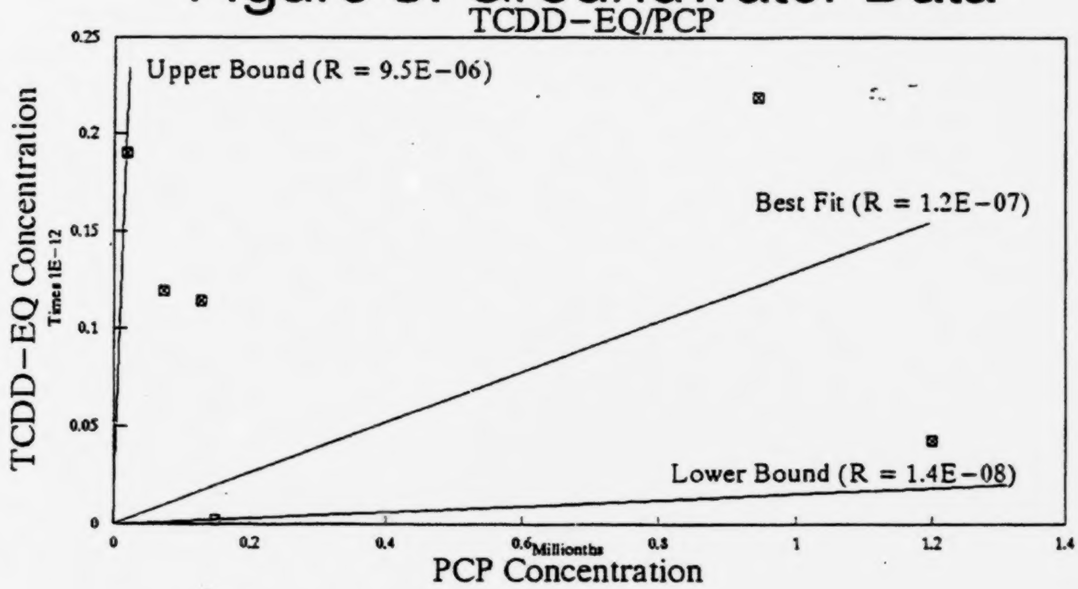
Notes:

*1: constituent phases of sample are listed in order of contribution to sample volume. The sample is classified as either oil, soil, or groundwater for correlation assessment based on the dominant phase.

Regression Output:

Constant	0
r Squared	0.00
No. of Observation	6
Best Fit R	1.2E-07

Figure 5: Groundwater Data



certainly increase the importance of this compound relative to the other congeners because of its enormous carcinogenic slope factor (q_1^*).

Comparison With Partitioning Predictions

The correlation constants generated from regression of field data can be compared with the ratios predicted using partitioning relations.

The concentration of a chemical i in oil is related to its equilibrium concentration in soil and water via the following coefficients:
equation (1)

$$K_{osi}=\frac{C_{oi}}{C_{si}}$$

equation (2)

$$K_{owi}=\frac{C_{oi}}{C_{wi}}$$

Dividing K_{osPCP} by $K_{osTCDD\text{-}EQ}$ and K_{owPCP} by $K_{owTCDD\text{-}EQ}$ and re-arranging yields equations (3) and (4), respectively:
equation (3)

$$\frac{C_{sTCDDEQ}}{C_{sPCP}}=\frac{K_{osPCP}}{K_{osTCDDEQ}}\times\frac{C_{oTCDDEQ}}{C_{oPCP}}$$

equation (4)

$$\frac{C_{wTCDDEQ}}{C_{wPCP}}=\frac{K_{owPCP}}{K_{owTCDDEQ}}\times\frac{C_{oTCDDEQ}}{C_{oPCP}}$$

Because contamination of soil and water at PCP sites originates from the oil phase, the ratio of TCDD-EQ to PCP in oil is used as input to this assessment (Best fit R = 1.2E-05). Values for K_{os} and K_{ow} are obtained from Jackson and Bisson (1990), whose landmark study generated two- and three-phase partition coefficients for PCP and PCDDs/PCDFs using weathered and fresh PCP oil (Table 6). The TCDD-EQ/PCP ratios for soil and water can be predicted by inserting these coefficients into equations (3) and (4):

equation (5)

$$\frac{C_{sTCDDEQ}}{C_{sPCP}}=\frac{10^{2.00}}{10^{1.74}}\times1.2\times10^{-5}=2.2\times10^{-5}$$

equation (6)

$$\frac{C_{wTCDDEQ}}{C_{wPCP}}=\frac{10^{2.35}}{10^{4.78}}\times1.2\times10^{-5}=4.5x10^{-8}$$

The ratio $K_{osPCP}/K_{osTCDD\text{-}EQ}$ is equal to 1.8. This implies that in a three-phase system at equilibrium, PCP favours the oil phase over soil to a slightly greater extent than PCDDs/PCDFs. Likewise, from $K_{owPCP}/K_{owTCDD\text{-}EQ}$, PCDDs/PCDFs favour the oil phase over water to a much greater extent than PCP.

The range of correlation constants for each medium is tabulated together with best fit and partitioning predictions in Table 7. From these values, it is apparent that the correlation constants approximate the partitioning-derived ratios for soil and water within factors of 2 and 3, respectively. Consequently, the correlation constants derived from data regression are somewhat verified in comparing well with the Jackson-Bisson coefficients.

Table 6. Three-Phase Partition Coefficients for Weathered PCP Oil (Jackson and Bisson, 1990)

Compound	Log K_{ow}	Log K_{os}
PCP	2.35	2.00
HxCDD	4.76	1.66
HpCDD	4.67	1.68
OCDD	4.75	1.64
HxCDF	4.91	1.86
HpCDF	4.72	1.75
OCDF	4.84	1.83

- **For PCDDs/PCDFs:**

$$\overline{K_{os}}=10^{1.74}$$

$$\overline{K_{ow}}=10^{4.78}$$

Table 7. Regression-Derived and Predicted TCDD-EQ/PCP Ratios

Medium	n	Range	Best Fit	Predicted
oil	8	2.0E-06 to 3.1E-05	1.2E-05	-*1
soil	11	1.1E-07 to 2.1E-04	1.2E-05	2.2E-05
water	6	1.4E-08 to 9.5E-06	1.2E-07	4.5E-08

*1: The TCDD-EQ/PCP ratio for oil was used as input in the partitioning calculations

ACKNOWLEDGEMENTS

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ASSESSMENT OF BIOCHEMICALS FOR THE CANADIAN ENVIRONMENTAL PROTECTION ACT.

N.D. Domey, Commercial Chemicals Branch, Environment Canada, Ottawa, ON, (819) 997-3204.

The Canadian Environmental Protection Act (CEPA) requires that all new substances be assessed for "toxicity" as defined by the legislation. Proponents of new substances for the Canadian market are required to provide detailed information and test data prior to the commencement of any manufacture or import. Assessment for potential adverse environmental and human health effects is a joint responsibility of Environment Canada and Health and Welfare Canada. The Departments must complete the assessment within a specified time period and, if the substance is suspected to be toxic, it must implement the appropriate controls.

Regulations are under development for the purpose of identifying data requirements and acceptable test methodologies for different substance categories (e.g., chemicals, polymers). Environmental fate and effects will be evaluated on the basis of intrinsic physical/chemical properties, environmental stability and compartmentalization and, most importantly, aquatic toxicity.

SERIAL DILUTION BIOASSAY FOR SEDIMENT TOXICITY USING HEXAGENIA MAYFLIES.*

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ABSTRACT

Studies of sediment toxicity are often not comparable because standardized reference sediments and test animals are unavailable. We used a synthetic reference sediment (42:42:16 w/w silica sand:sculptor's clay:potting soil) to dilute highly contaminated Detroit R., MI sediment. Laboratory-reared larvae were subjected to either 21-d or to lifetime (244-d) exposures of a sediment dilution series (1:0, 1:1, 1:3, 1:7, 1:15 or 0:1 contaminated:reference). Over 244 d, complete mortality occurred in the 1:0 mixture. Adults emerged only from dilutions $\geq$1:3. However, effects of contaminated sediment on 21-d survival and growth of large *Hexagenia* were not significant. Thus, contaminant-induced mortality was not an acute response. Gas chromatographic analysis of organochlorine contaminants in sediments, larvae and emerging adults indicated significant compound-specific variability in dilution ratios and patterns of uptake. Contaminant uptake patterns by larvae among dilutions in short-term exposures were markedly nonlinear: strongly hydrophobic compounds were underrepresented in larvae reared in the most contaminated dilution. Lifetime-exposure larval: sediment and adult: sediment contaminant ratios were compound-specific but constant among dilutions. These important data can be used to directly infer sediment contamination levels from field-collected organisms. Parallel serial dilution sediment tests conducted on other benthic indicator organisms could considerably enhance the value of indicator organisms in reflecting sediment conditions.

1. INTRODUCTION

Various bioassay and biomonitoring techniques assume that variation in test organism survival, growth and/or ultimate contaminant burden directly reflect the concentration and toxicity of contaminants in the sediment. Response differences between contaminated and uncontaminated site-collected treatments is assumed to reflect the influence of the contaminants alone. We have proposed that comparing both field-collected reference and contaminant-influenced sediments with a standard, laboratory-prepared control permits one to ascertain those test organisms' responses to on-site sediments that result from inappropriate or suboptimal physical sediment characteristics (Ciborowski and Corkum 1989).

We have been evaluating the utility of synthetic sediment as a reference (control) substrate in short-term (21-d; Ciborowski et al. 1991) and long-term (hatchling to emergence; 244-d) sediment bioassay procedures using *Hexagenia* mayfly larvae. Detailed results of the 21-d study were reported by Ciborowski et al. (1991). Herein, we report the results of the long-term study.

2. MATERIALS AND METHODS

Our study involved laboratory comparison of *Hexagenia* growth and survival from newly-hatched larvae to adults in sediment highly contaminated with organochlorine compounds (collected from the Trenton Channel of the Detroit R., MI; site 107 of Furlong et al. (1988)) that had been diluted by addition of various quantities of a laboratory-mixed standard reference sediment (STND3; a 42:42:16 w/w mixture of silica sand, sculptor's clay, and potting soil, respectively). Detailed methodology for sediment collection and preparation, and experimental procedures is described by Ciborowski et al. 1991, 1992).

Dilutions of contaminated sediment were prepared by combining known proportions (by dry mass) of Trenton Channel sediment (loss on ignition (LOI) 10.6%) with STND3 sediment (LOI 8.4%) to produce 6 mixtures, designated 1X, 0.5X, 0.25X, 0.125X, 0.063X and 0X, where 1X represents 100% Trenton Channel sediment and 0X represents 0% Trenton Channel sediment (= 100% STND3 sediment). These mixtures corresponded to Trenton:STND3 ratios of 1:0, 1:1, 1:3, 1:7, 1:15 and 0:1, respectively. Five replicate samples of each treatment were prepared by placing 350 mL of sediment within 2-L Universal glass jars, topping up with dechlorinated water, and aerating. Jars were kept at room temperature (20-22°C) and 16:8 L:D photoperiod.

One-day-old *Hexagenia limbata* larvae (hatched from cold-stored eggs acquired from females collected at L. St. Clair) were randomly assigned to experimental sediments (15 per jar). Larvae were fed twice weekly with 2 mL of a mixture of Tetramin®, baker's yeast and alfalfa powder (M.G. Henry, Minnesota Fish Coop, Univ. Minnesota, pers. comm.). Tanks were monitored at feeding times for emerging adults for the first 150 d, and daily thereafter. Adults were frozen and stored for contaminant analysis.

The experiment was terminated after 244 d. Water was carefully siphoned from containers and replaced with a 4-cm depth of carbonated water (an anaesthetic) in a manner that minimized disturbance to the sediments. The Larvae floated to the surface of the carbonated water within 20 min. Larvae were removed to a petri plate, measured, and frozen. Sediment was transferred into a hexane-rinsed, 500-mL amber sample jar and stored frozen.

Triplicate sediment samples, duplicate (21-d) or single pooled (244-d) *Hexagenia* larval samples, and single pooled *Hexagenia* adult samples were analyzed for organic contaminants concentration in the Great Lakes Institute analytical laboratory (Hewlett-Packard model 5790A GC with 25 m x 0.25 mm fused silica column and electron capture detector). Concentrations of 19 contaminants (14 PCB congeners, total PCBs, DDE, QCB, OCS, HCB, trans-nonachlor) were quantified based on peak patterns and comparison to those in standard mixes of known concentration. Sediments were Soxhlet extracted for 24 h and analyzed according to procedures described by Gobas et al. (1989). *Hexagenia* larvae or adults were homogenized with mortar and pestle and extracted by solid-liquid column extraction using 20 g anhydrous sodium sulphate and 300 mL 50% dichloromethane-50% hexane mixture as the solvent (Kovats and Ciborowski 1989). Contaminant concentrations were expressed as mg/kg organic carbon (sediments) or mg/kg lipid (mayflies).

3. RESULTS AND DISCUSSION

3.1. *Hexagenia* Growth and Survival

Little mortality occurred during the short-term (21-d) study, conducted with half-grown larvae (Ciborowski et al. 1991). Growth in that experiment ranged from a mean loss of 3% of head width to an increase of 11% within individual jars. There were no significant differences in mean growth among treatments, although growth appeared to be stimulated by sediments that contained 0.125X or more Trenton Channel Sediment (Ciborowski et al. 1992).

First emergence of a subimago in the long-term study occurred from a 0X container after 125 d. Next emergences were recorded after 180 d or later. However, there were no clear trends in the pattern of emergence among treatments. Adults emerged from containers containing up to 0.25X Trenton Channel sediment. Emergence of at least one adult was observed from 8 of the 30 jars. No individuals emerged from any of the 0.5X or 1.0X dilutions. Although there was no significant difference in either the mean number of adults emerging per tank, or the mean number of replicate tanks per dilution yielding emerging adults (regression, $p > 0.05$), the probability that none of the 0.5X or 1.0X tanks would yield adults by chance was <0.05 (binomial theorem, $p = 0.045$). Between 1/4 and 3/4 of surviving animals in any single replicate tank had emerged by the end of the experiment.

Overall lifetime survival in tanks was quite low (25-35% in containers with some survival). Complete mortality occurred in tanks from the control (0X dilution) as well as from treatments containing dilutions of Trenton Channel sediment. Only 12 of the 30 replicate tanks had emergences of adults or still retained larvae at the end of the experiment. The mean number of survivors tended to be higher in less contaminated treatments, but there was no significant linear trend in larval survival with respect to degree of sediment contamination (regression $p > 0.05$). However, only one of the 10 tanks containing the most contaminated (0.5X or 1.0X Trenton Channel) sediments supported surviving individuals (a single larva in a 0.5X replicate). The probability of this occurring by chance was <0.05 (binomial theorem, $p = 0.040$). There was no significant difference in mean head width of larvae remaining in containers at the end of the experiments (regression, $p > 0.05$).

In general, our long-term data suggest that since emergence occurred only from sediments diluted by a factor of 3:1 (0.25X) or more, overall contaminant levels in Trenton Channel sediments are on the order of four times too high to permit successful completion of the life cycle of *Hexagenia*.

3.2. Contaminant Uptake

Trenton Channel sediments (1.0X) were highly contaminated with all compounds assayed. Levels of contamination of STND3 sediment were low or nondetectable, with concentrations equivalent to those found in sediments from reference areas that receive minimal inputs of aerial deposition of organic contaminants. Larvae reared in reference (0X) sediment had very low but detectable concentrations of all compounds assayed except OCS over both short-term and long-term rearing intervals (Ciborowski et al. 1992).

Patterns in 21-d contaminant uptake by *Hexagenia* with respect to specific contaminants paralleled the differences in concentration ratios between 1X and 0.063X sediment dilutions (Ciborowski et al. 1991). Concentrations of QCB and HCB in *Hexagenia* increased approximately log-linearly with increasing proportion of Trenton Channel sediment (Figure 1A). Regression analysis revealed a significant positive relationship between *Hexagenia* concentration of QCB and HCB and Ln (Proportion of Trenton Channel sediment ($F=255$, $p << 0.001$, $R^2 = 0.94$). However, there was significant deviation from linearity ($p < 0.005$). Concentrations of PCBs increased log-linearly with increasing proportions of Trenton Channel sediment for all dilutions except the 1X treatment (Figure 1B; linear regression analysis, $F = 24.45$, $p < 0.05$, $R^2 = 0.84$).

These findings are consistent with a differential uptake model of bioconcentration and bioaccumulation. Relatively soluble compounds (Log Kow < 6.0) are thought to be taken up primarily by transport across respiratory surfaces, whereas ingestion pathways are more important for the less soluble compounds (Landrum and Poore 1988, Bedard 1990). We suspect that the reduction in PCB uptake in 1X treatments reflects a change in either feeding or physiological/metabolic behaviour of larvae. However, no significant differences in growth were found among treatments, so that these differences cannot be attributed to feeding rate per se. Accordingly, differential binding of contaminants to the carbon species present in Trenton Channel sediment is a more tenable explanation for these differences.

Patterns of contaminant uptake over 244 d with respect to sediment dilutions were remarkably similar to patterns observed over the 21-d interval. Correspondence was closer for the less hydrophobic compounds (e.g., HCB, Figure 1A) than for the more hydrophobic compounds such as the PCBs (Figure 1B).

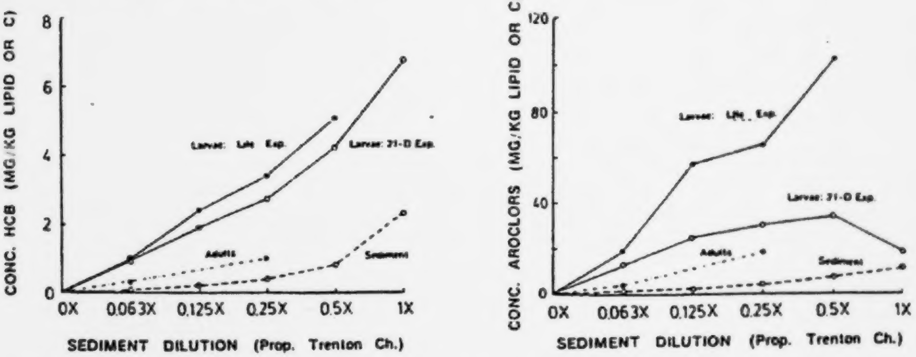

Figure 1. Mean (±1 SE) concentration of HCB (A) and total PCBs (B) in sediment and *Hexagenia limbata* larvae and adults after 21 and 244 d in sediment mixtures ranging from pure (1X) Trenton Channel sediment to pure (0X) STND3 sediment. Standard error bars are less than the diameter of the points. Open points and dashed line, sediment after 244 d; solid points and dotted-and-dashed line, adults after 244 d; open points and solid line, larvae after 21-d; solid points and solid line, larvae after 244 d.

Larval BAFs of HCB determined over 244 d were only slightly greater than the 21-d BAFs, indicating that this compound achieves equilibrium between larvae and sediments fairly rapidly. The BAF ratio was constant over all sediment dilutions (lines for larvae and adults are almost exactly parallel; Figure 1A). The slow equilibration rate of PCBs (expressed as total Aroclors, Figure 1B) was reflected in the much higher BAFs of larvae exposed to sediment treatments for 244 d than when exposed for only 21 d. Again, BAF appeared to be relatively constant across all sediment dilutions in which some larvae survived over the 244-d interval of the experiment.

When *Hexagenia* larvae transform into adults, their body mass decreases dramatically, primarily due to reduced water content. The larval cuticle (chitin) is shed, but almost all body lipid is retained during the transformation. The result is a nine-fold increase in the relative lipid content of adults as compared to larvae. This results in a 2-3 fold increase in the contaminant concentration when expressed on a unit per fresh mass basis, but a marked decrease in concentration when expressed on a unit per unit lipid basis (Figure 1). The BAFs for adult *Hexagenia* were >1.0 for all compounds examined when expressed on a mass per unit lipid/mass per unit organic carbon basis. Thus, *Hexagenia* mayflies are unquestionably able to biomagnify contaminants over the course of their lifetime. The magnitude of BAF for adult *Hexagenia* appeared to be constant across various sediment dilutions (data available for only 0X, 0.063X and 0.25X treatments) and increased with increasing hydrophobicity of specific contaminants. These trends are consistent with expectations of first-order toxicokinetics.

Our data from the long-term study will be especially valuable in establishing *Hexagenia* as a 'calibrated' indicator of contaminant conditions in natural habitats. Our BAF ratios can be used to directly estimate the likely sediment concentrations of individual compounds in regions from which adults and mature larvae are collected.

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AHH INDUCTION IN RAINBOW TROUT BY CHLORINATED DIPHENYL ETHERS. C.D. Metcalfe, ERS Program, Trent University, Peterborough, ON, (705) 748-1272, and A.J. Niimi, Fisheries and Oceans, CCIW, Burlington, ON.

The relative potencies for induction of hepatic aryl hydrocarbon hydroxylase (AHH) activity in rainbow trout *Oncorhynchus mykiss* were determined for four polychlorinated diphenyl ethers (PCDEs) and 2,3,7,8-tetra-chlorodibenzofuran (2,3,7,8-TCDF). Two of the PCDEs did not elevate hepatic AHH activity, but 3,3', 4,4'-tetraCDE and 3,3', 4,4', 6-pentaCDE induced elevated AHH, with ED50s for this response calculated as 53.98 and 5.49 µmol/kg respectively. The ED50 for 2,3,7,8-TCDF was 0.57 µmol/kg. AHH-toxic equivalency factors (AHH-TEFs) for these compounds, calculated in relation to the AHH induction potency of 2,3,7,8-TCDD in rainbow trout, were 0.012, 0.0013 and 0.00013 for 2,3,7,8-TCDF, 3,3', 4,4', 6-pentaCDE and 3,3', 4,4'-tetraCDE, respectively. AHH-toxic equivalent quantities (AHH-TEQs) calculated for fish from Whitby Harbour, Lake Ontario are greater for the PCDEs than for 2,3,7,8-TCDF. These results will be discussed in relation to the concentrations of PCDEs detected in fish from industrially contaminated sites in the Great Lakes.

SEDIMENT TOXICITY ASSESSMENT - NORTH SASKATCHEWAN RIVER.

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Abstract

Surface aquatic sediments were collected from sites along the North Saskatchewan River near a storm-sewer discharge containing wood-preserving chemicals. Water extracts of 24 samples were analyzed using nematode, Microtox and seed germination bioassays. Selected samples were also extracted with methanol and the analyses repeated. Pentachlorophenol (PCP) analysis was conducted on 16 sediment samples and on samples of overlying river water. Microtox and nematode maturation displayed the greatest sensitivity to the water and methanol extracts. Samples taken nearest the storm-sewer and containing PCP yielded the highest measures of toxicity. Generally, methanol extracts were more toxic than water extracts. Extracts of sediments from some sites with no known or suspected source(s) of toxicants also caused toxic responses in several bioassays.

Introduction

In 1990, the Water Quality Branch of Saskatchewan Environment and Public Safety (SEPS) initiated a study to evaluate various bioassays as monitoring protocols for assessing the acute and chronic toxicity of industrial and municipal effluents. Part of that study also involved the evaluation of bioassays for assessing the toxicity of aquatic sediments, particularly those near effluent discharges.

The main purpose of the study was to evaluate the sensitivity of three bioassays for detecting toxicity in sediments affected by storm-sewer discharges containing wood-preserving chemicals as well as in sediments not exposed the storm-sewer effluent or to other apparent pollution sources.

The following presentation describes the results obtained using nematode, Microtox and seed germination bioassays to analyze aquatic sediments from the North Saskatchewan River.

Site Description

Water quality and hydrological characteristics of the North Saskatchewan River in the reach where sediment samples were obtained are described in a previous report (Saskatchewan Environment 1984). The toxicological characteristics of the storm-sewer effluent are described in a previous report related to effluent toxicity evaluation and bioassay assessment (Saskatchewan Environment and Public Safety 1991).

The 8th Avenue East storm-sewer was the effluent source of primary interest in this study. The storm-sewer drains a former wood-preserving site and is known to carry creosote and other wood-preserving chemicals, including pentachlorophenol (PCP), to an outfall on the south shore of the North Saskatchewan River within the City of Prince Albert.

Materials and Methods

Twenty-four sediment samples were collected from 19 locations along the shoreline of the North Saskatchewan River. Samples were obtained at sites upstream, downstream and across the river from the outfall of the 8th Avenue East storm-sewer in the City of Prince Albert. The sediment samples were comprised of the upper 1 to 5 cm of surface sediment and were collected using a stainless steel scoop or were scraped directly into a metal sampling container.

Selected samples were mixed well and portions were retained for PCP analyses and for toxicity assessment. Samples of river water were also obtained for PCP analyses.

Eighteen river water, 8 storm-sewer effluent, and 16 selected sediment samples were analyzed for PCP in Calgary by Enviro Test Laboratories.

Water extracts of each sediment sample were used as test material for three bioassays. The bioassay protocols used to test water extracts included the nematode (*Panagrellus redivivus*) survival, growth, maturation test of Samoiloff (1990), the Microtox bacteria (*Photobacterium phosphoreum*) luminescence test, and the lettuce seed (*Lactica sativa*) germination and root elongation test. Detailed protocol descriptions are described in literature provided to clients by the two analytical laboratories, BioQuest International (Winnipeg, Canada) and HydroQual Laboratories Limited (Calgary, Canada), contracted to perform sample analyses.

Water extracts were tested at respective concentrations of 10%, 2.8 - 45.4% and 100% (V/V) in the nematode, Microtox and seed germination tests.

Methanol extracts of selected sediment samples were also prepared according to a protocol described by Samoiloff (personal communication) and documented in protocols printed by Bioquest International.

Methanol extracts from 12 selected sediment samples were analyzed at a methanol concentration of 3% (V/V) by the nematode test and at 1% (V/V) in the Microtox test. Seven of these methanol extracts were also analyzed at a 10% (V/V) concentration using the seed germination/root elongation test.

Results and Discussion

PCP was detected in the 8th Avenue East storm-sewer effluent discharge samples at concentrations ranging from 330 to 660 $\mu g.L^{-1}$. In only three surface water samples taken near the mouth of the storm-sewer and at sites as for downstream as 50 m below the storm-sewer outfall was PCP detected. In these three samples the PCP concentration ranged from 1.3 to 2.1 $\mu g.L^{-1}$. PCP was not detectable in river water samples obtained at any site upstream or across the river from the 8th Avenue East storm-sewer. One river water sample taken 23 km downstream of this storm-sewer contained a trace level (eg. 0.12 $\mu g.g^{-1}$) of PCP.

PCP was detected in 9 of 16 sediment samples. All nine of these samples were from the shoreline area within 50 m of the storm-sewer outfall. Concentrations of PCP in these samples ranged from 0.36 $\mu g.g^{-1}$ at the outfall to 0.079 $\mu g.g^{-1}$ 50 m downstream of the outfall. PCP was not detected in sediments upstream of the outfall.

These results indicated that PCP contamination of the river was confined to areas near and downstream of the 8th Avenue East storm-sewer outfall.

In order to assess the sensitivity of the nematode, Microtox and seed germination protocols in detecting toxicity in water extracts and methanol extracts the frequency of occurrence of significant measures of response were determined for each test protocol. The results are summarized in Figures 1 and 2.

For the nematode test a significant toxic response to the 10% water extract was considered to have been observed in test cultures when 90% or less of the test cultures failed to survive, to grow, or to reach maturity. In the Microtox test, a significant response was considered to have occurred when the EC_{50} value was reported for a water extract concentration of 90% or less. For seed germination and root growth (elongation) significant toxicity was considered to have been observed when 90% or less of seeds germinated (relative to control cultures) or when root growth was 90% or less of that observed for control cultures.

From the summary of results shown in Figure 1 it is apparent that the most frequent detections of presumed toxic response were with the nematode maturation and Microtox tests. In these two tests significant toxicity was detected in 64% and 50% of the water extracts examined, respectively. None of the extracts tested were acutely toxic to nematodes and in only 11% of the extracts was significant inhibition of nematode growth observed. Significant inhibition of seed germination and root growth were observed in 38% and 25% of the water extracts, respectively.

Amongst the 24 water extracts examined four were derived from sediments obtained upstream of any obvious contaminant source, and in which PCP was not detected. Toxic responses were detected by nematode maturation in one of these samples and by Microtox and the root elongation tests in one other sample and by seed germination in a third sample.

Eleven selected sediments from which water extracts had been prepared were also used to prepare methanol extracts. All eleven methanol extracts were tested using the nematode and Microtox procedures. Seven of these were also tested using the seed germination/root elongation bioassay. Significant toxicity associated with these extracts was considered to have been observed with the 3% (V/V) extracts in nematodes when 90% or less of the nematodes survived, grew or matured. EC50 values of 90% or less from the Microtox test using 1% methanol extracts, and seed germination and root elongation (using 10% methanol extracts) occurring at 90% or less of that of control cultures, were considered indicative of toxic responses.

The frequencies of occurrences of presumed toxic responses observed with the three bioassays applied to sediment methanol extracts are shown in Figure 2.

Figure 1. Frequency - Toxic Responses - Water Extracts

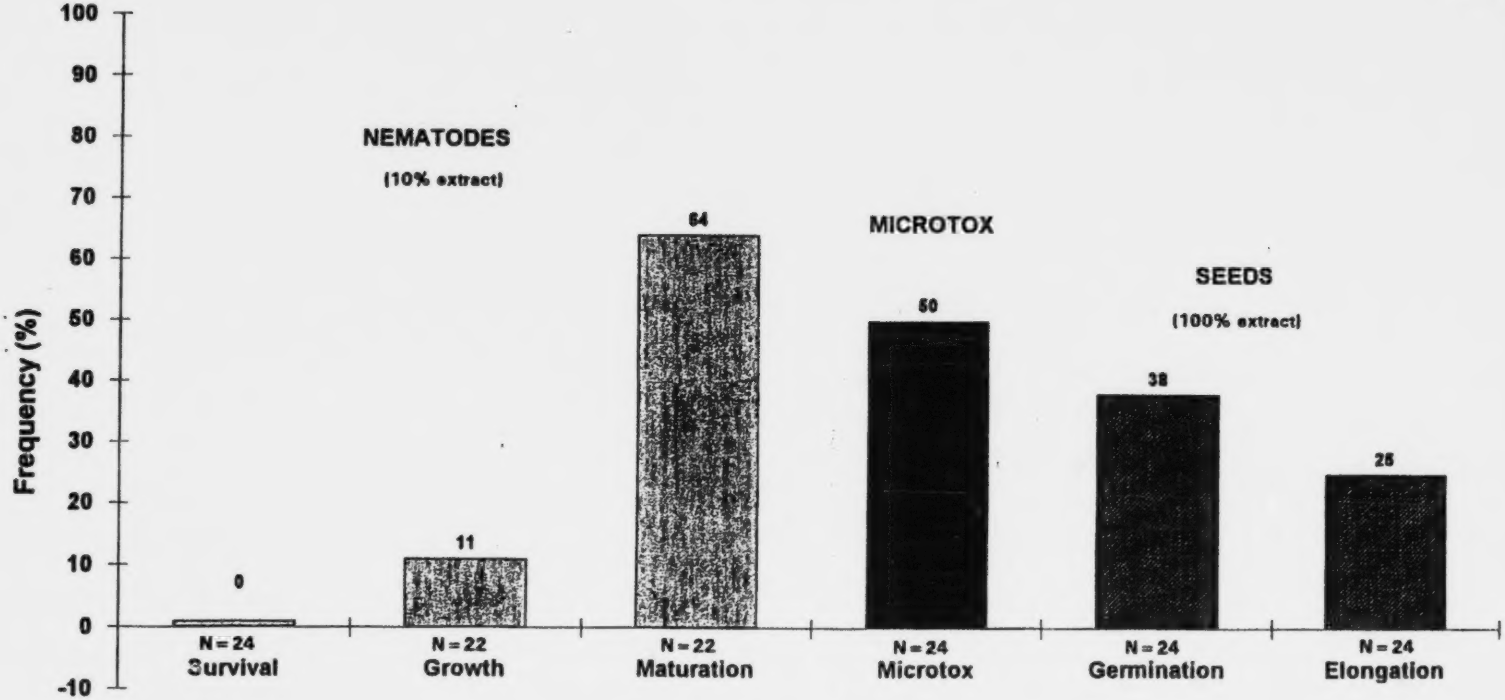
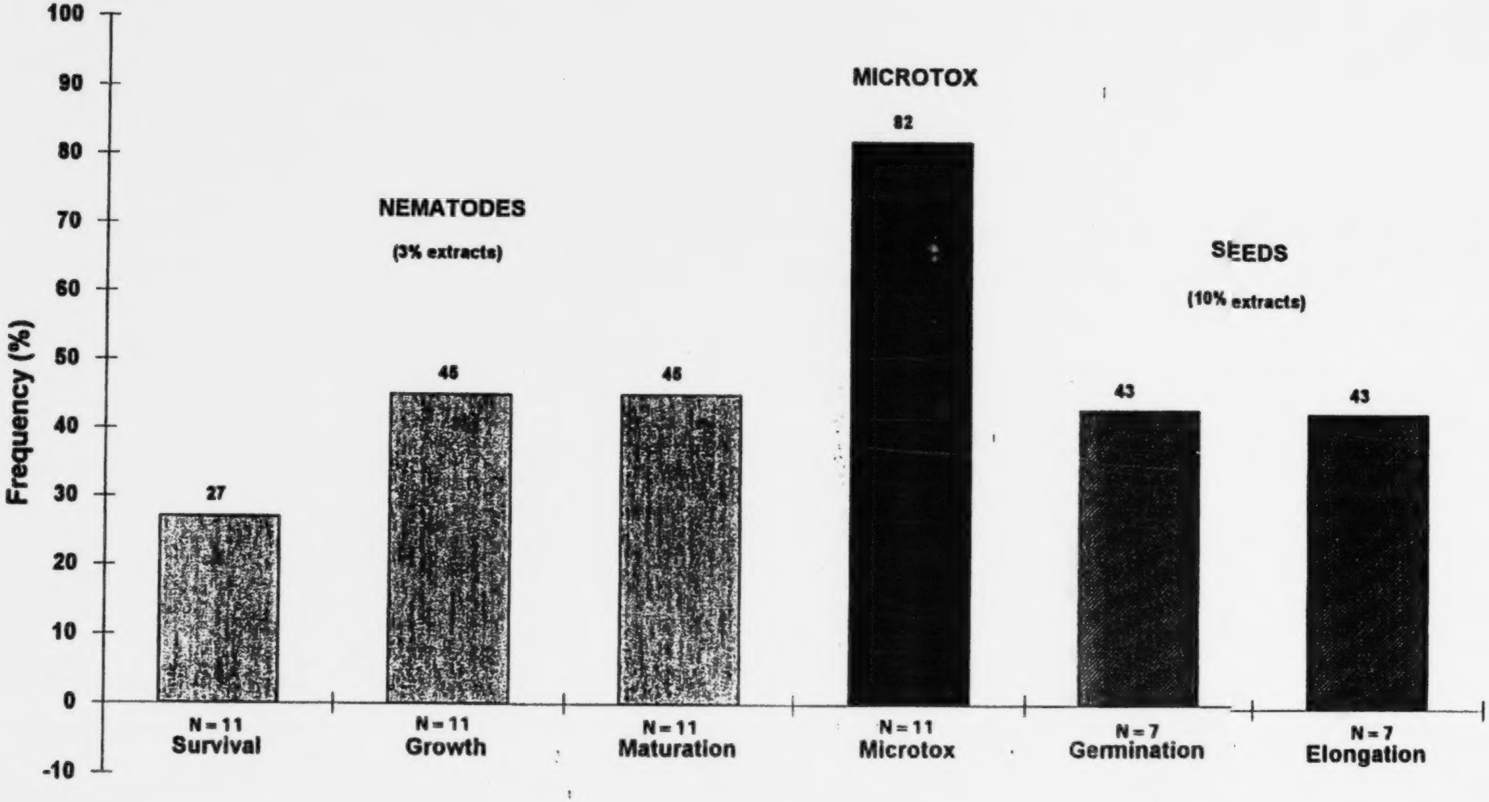


Figure 2. Frequency - Toxic Responses - Methanol Extracts



The results shown in Figure 2 indicate that the Microtox test applied to 1% methanol extracts yielded the highest frequency of toxic responses (82%) in the eleven extracts examined. Nematode growth and maturation were each significantly inhibited at a frequency of 45% while survival was significantly reduced in only three (27%) of these extracts.

Only 7 methanol extracts were tested using the seed germination/root elongation procedure. Three of these extracts displayed significant inhibition of both germination and root growth.

As had been observed with water extracts of sediments from four locations without obvious contamination and without detectable PCP toxic responses were detected in each of the four methanol extracts from these sites. Each methanol extract elicited a significant toxic response in at least one of the test procedures used for analyses.

In general, methanol extracts yielded results indicative of greater toxicity than was observed for the water extracts from the same sediment samples. However, additional analyses and replication would be required to confirm this trend for the sites examined in this study.

The most extreme measures of toxicity were observed for methanol extracts obtained from four sediment samples in which PCP was measurable at concentrations of 0.23 to $0.36/\mu g.g^{-1}$. Each of these sediments was collected from sites that were downstream and within a 10 m distance from the storm-sewer outfall.

The observation of some toxicity in water and methanol extracts of sediments from areas with no apparent contamination cannot be readily explained. Ongley et al. (1988), in their toxicological assessment of suspended sediments in the North Saskatchewan River near Prince Albert, also reported toxic responses in sediment extracts in the absence of detectable priority pollutants. It may therefore be prudent to determine the causes of toxicity at sites with no obvious contamination before the three bioassays examined in this study are adopted as protocols for use in delineating areas of sediment contamination.

Acknowledgements

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* Reports are available from Saskatchewan Environment and Public Safety, 3085 Albert Street, Regina, Saskatchewan, Canada, S4S OB1.

HEPATIC MICRONUCLEI IN FISH AS A BIOMONITOR FOR GENOTOXIC CHEMICALS IN THE AQUATIC ENVIRONMENT.

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We recently developed an *in vivo* genotoxicity assay with teleosts in which frequencies of micronuclei in hepatocytes are elevated when fish are exposed to known genotoxic chemicals. In order to test the suitability of this test as a biomonitor for genotoxic chemicals, we collected English sole from the Duamish Inlet; a highly contaminated area of Puget Sound. Sole from this region had elevated micronucleus frequencies of 5.8 micronuclei per 1000 hepatocytes in comparison to frequencies of 0.7 micronuclei per 1000 hepatoctyes in sole from a pristine Reference site in Puget Sound (Useless Harbour). Duamish fish also showed high prevalences of hepatic lesions, and high levels of hepatic DNA adducts. These data indicate that the teleost hepatic micronucleus assay is a relatively simple and sensitive biomonitor for genotoxic chemicals in the aquatic environment.

CHIRONOMID LARVAE AS POTENTIAL MONITORS OF SEDIMENT GENOTOXICITY.*

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ABSTRACT

Chironomids are acknowledged as potentially important indicators of the effects of sediment-bound contaminants. Sediment bioassays can indicate toxic effects on survival and growth. Antennal or mouthpart deformities in natural populations suggest sediment teratogenicity. Our laboratory is adapting two chromosomal visualization techniques for use with chironomids to provide a direct assay for sediment genotoxicity. Chironomids, like many other Diptera, possess giant polytene chromosomes in their salivary glands. Banding patterns can be used to characterize genetically unique but morphologically indistinguishable populations. Exposure of larvae to toxic contaminants may be reflected as chromosomal breaks, or in changes in the frequency of puffs (swellings) at possibly characteristic locations on specific polytene chromosomes. We are also attempting to differentially stain chromosomes in mitotically active diploid tissue (wing bud and neural) to assess frequencies of sister chromatid exchanges (SCEs). SCEs are more common in tissues exposed to mutagenic agents. Successful application of these techniques to chironomids could yield an animal that may be used to simultaneously indicate toxic stress (puffing frequencies), teratogenicity (incidence of deformities) and mutagenicity (SCE frequency) of sediments.

INTRODUCTION

Recognition of freshwater sediments as a site of retention of persistent chemicals has stimulated development of diverse methodology for assessing sediment toxicity (e.g., Nebeker et al. 1984, Giesy et al. 1988, ASTM 1990, Bedard et al. 1992). The acute toxic effects of highly contaminated sediments are quickly manifested through mortality of test organisms. Standard assays for chronic effects involve detecting changes in growth and reproduction (Geisy et al. 1988, Borgmann and Munawar 1989, Reynoldson et al. 1991, Bedard 1992). However, a major concern of the effects of 'in place' pollutants is the association of sediment-bound contaminants with genotoxic effects, for which relatively few suitable assays are yet available. Benthic aquatic invertebrates are often test organisms of choice because they live in direct contact with the sediments, are amenable to laboratory culture, and grow rapidly.

Among the suite of benthic invertebrates used in sediment toxicity assessment, larval midges (Diptera: Chironimidae) are especially useful because they have a short generation time and are broadly distributed among both pristine and contaminated habitats. Laboratory sediment bioassay procedures have been proposed for *Chironomus tentans* that are sensitive to both acute and chronic toxic effects (Nebeker et al. 1984, Giesy et al. 1988, Bedard et al. 1992). Additionally, Warwick (1985, 1988, 1991) has conducted extensive work linking degree of in situ sediment contamination with morphological deformities in chironomid heads, indicative of teratogenic and possibly genotoxic effects. Similar correlations have been reported by Dickman et al. (1989) and Dermott (1991).

Our laboratory is evaluating two potentially useful chromosomal visualization techniques, salivary gland polytene chromosome preparation, and sister chromatic exchange (SCE) for use with chironomids to provide a direct assay for sediment genotoxicity. We believe there is great value in being able to employ a single test organism that could be used to provide simultaneous measures of a broad range of routes of toxicity.

POLYTENE CHROMOSOMES

Larvae of chironomids, like many other Diptera that produce copious salivary secretions, possess giant polytene chromosomes in their salivary glands. Mitosis does not occur in salivary gland cells. Instead strands of DNA align to produce giant chromosomes whose detailed structure can be seen by light microscopy. Consistent sequences of light and dark bands are species specific and provide systematicists with a powerful tool for discriminating morphologically identical but genetically incompatible populations (sibling species). Cytotaxonomy has been well developed to distinguish European chironomid species (e.g., Michailova and Petrova 1991). Satellite chromosomes (fragments of a polytene chromosome that become broken away from the main chromosomal body) can occasionally be seen in preparations (P.H. Adler, Clemson University, personal communication). We are investigating whether incidence of such fragments may be associated with especially high exposures of mutagens during early larval development.

Polytene chromosomes also characteristically exhibit puffs, broadened sections of chromosomes. In fruit flies, transitory puffs in specific regions have been associated with periods of rapid protein synthesis related to specific developmental phases of the larvae. Appearance of puffs is also associated with production of heat-shock proteins following application of stress (e.g., Jang 1992). Exposure of larvae to toxic contaminants may also possibly be reflected in changes in the frequency of puffs at specific locations on chromosomes. In *Chironomus* larvae, puffs can occur following exposure to some heavy metals (R. Tonguay, Laval University, personal communication). Localization of such characteristic markers could provide an important tool useful in assessing chronic contaminant stresses.

We are examining preparations made from larvae collected from 6 sites along the Detroit and St. Clair Rivers using acid fuschin staining (Rothfels and Dunbar 1953). Many larvae collected during warm-water periods in summer have small salivary glands, whose chromosomes provide poor resolution. Our preliminary investigations suggest that polytene chromosome examinations might be most suitable for laboratory bioassay applications.

SISTER CHROMATID EXCHANGES

Sister chromatid exchange (SCE) occurs during the S-phase of the cell cycle of mitotically active cells, when DNA replication is occurring (Zakharov 1982). It involves a reciprocal exchange between portions of DNA double helices at identical chromosomal loci in the presence of a mutagenic chemical. The mechanism of SCE is thought to reflect the ability of a cell to replicate DNA on a damaged template and continue the process of cell division (Kligerman et al. 1981). Sister chromatid exchanges can be detected by differentially staining the two chromatids in vivo.

A strong association has been documented between incidence of SCEs and exposure to mutagenic substances in many vertebrates (Tice and Hollaender 1984). The technique has been successfully applied to larvae of marine worms (Pesche et al. 1981) and mussels (Harrison and Jones 1982). However, previous attempts to induce SCEs in insects have had limited success (Gatti et al. 1979).

We have examined several tissues of larval *Chironomus* for incidence of SCEs using a modification of the methods of Pesche et al. (1981) and Nayak and Petras (1985). Tissues of the thorax, somatic musculature and intestinal epithelium are unsuitable for SCE induction because the cells become arrested in late metaphase. However, we have been able to isolate nuclei with appropriate chromosomes from wing bud tissue and cephalic (neural) tissue of late instar larvae. The small karyotype of chironomids (n=4) should make this organism especially amenable to rapid evaluation of (possibly site specific) levels of SCE induction.

Laboratory sediment bioassay experiments are in progress that will better enable us to evaluate the utility of polytene chromosome analysis and SCE induction. We anticipate establishing correlations among sediment contaminant concentrations and levels of genotoxicity. We will also determine the degree of correspondence between these measures of toxic stress and other, more conventional methodologies (incidence of deformities, growth inhibition).

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INFLUENCE OF BIOLOGICAL FACTORS ON THE BIOACCUMULATION OF METALS BY *Elliptio complanata* AND *Lampsilis radiata* (Bivalvia: Unionidae) FROM THE ST. LAWRENCE RIVER.

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Studies on marine mussels have shown that biological factors can significantly influence the bioaccumulation of metals by these organisms, but similar studies on freshwater mussels are virtually absent. This information is needed for the proper design of mussel monitoring programs in freshwater systems. *E. complanata* and *L. radiata* specimens of a wide size range were collected from a metal-polluted site downstream of Sorel, Quebec; 35 females and 35 males (gravid/non-gravid for *E. complanata*) of each species were weighed, measured, aged and analyzed individually for residues of 12 metals in their soft tissues. The best-fitting multiple regression models predicting metal concentrations from these variable were determined for each metal and species. For *E. complanata*, sex was a significant predictor ($p = 0.05$) for Cu, Mn, and Se, whereas size was highly significant ($p = 0.01$) for Zn and Mn, and significant for Se. Age was significant or highly significant for all metals except Ni and Pb, indicating the importance of standardizing this factor in biomonitoring programs. Concentrations of all metals except Al were higher in older specimens. Results for *L. radiata*, which has a faster growth rate, shorter life span and separate sexes, will be compared with *E. complanata*.

WHAT IS THE POLLUTION STATUS OF NORTH SEA SEDIMENTS?

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ABSTRACT

An international sea-going workshop (March 1990, Bremerhaven, Germany) provided the opportunity to conduct detailed sediment toxicity testing in concert with studies of fish histopathology, bioaccumulation, benthic community structure and sediment chemical contamination. Two gradients of sediment chemical contamination were tested: one from an abandoned oil platform, and the other from the mouth of the Elbe River northward, to the Dogger Bank. Using a preponderance of evidence approach, it was determined that sediments nearest the Elbe are moderately polluted (pollution is defined as contamination, toxicity and community alteration) and that sediments offshore and at the Dogger Bank are unpolluted. Sediments nearest the oil platform (0m) showed evidence for a low level of pollution, but there was no evidence of pollution 125m from the platform (no stations were located between 0 and 125m). The results of this study suggest the testable hypothesis that North Sea sediments away from point sources of pollution (e.g., coastal areas, drilling platforms) are presently not polluted.

INTRODUCTION

In March 1990, as part of an international seagoing workshop (International Council for the Exploration of the Sea [ICES] Working Group on the Biological Effects of Contaminants and International Oceanographic Commission [IOC] Group of Experts on the Effects of Pollution), a variety of sediment assessment methods were applied to two gradients of expected chemical contamination in the North Sea (Figure 1). One gradient comprised seven stations at increasing distances down-current from an abandoned oil-drilling site off the Dutch coast (Drilling Area, DA). The other gradient comprised nine stations at increasing distances from the mouth of the Elbe River, Germany, out to the international waters of the Dogger Bank (German Bight, GB). The overall aim of the workshop was to compare available biological effects monitoring techniques for marine pollution.

The variety of sediment assessment methods used allowed for an integrative assessment (Chapman et al., 1992) of the pollution status of the tested sediments, and an extrapolation to include North Sea sediments in general. In particular, the Sediment Quality Triad approach and concept was applied to sediment toxicity, chemical contamination and benthic infaunal community structure data. The Triad is an effects-based approach to describing sediment quality (Long and Chapman, 1985; Long, 1989a; Chapman, 1986a, 1989; Chapman et al., 1987a,b, 1991a,b; Alden, 1992; Cross et al., 1991), which is described in detail by Chapman (1990, 1992).

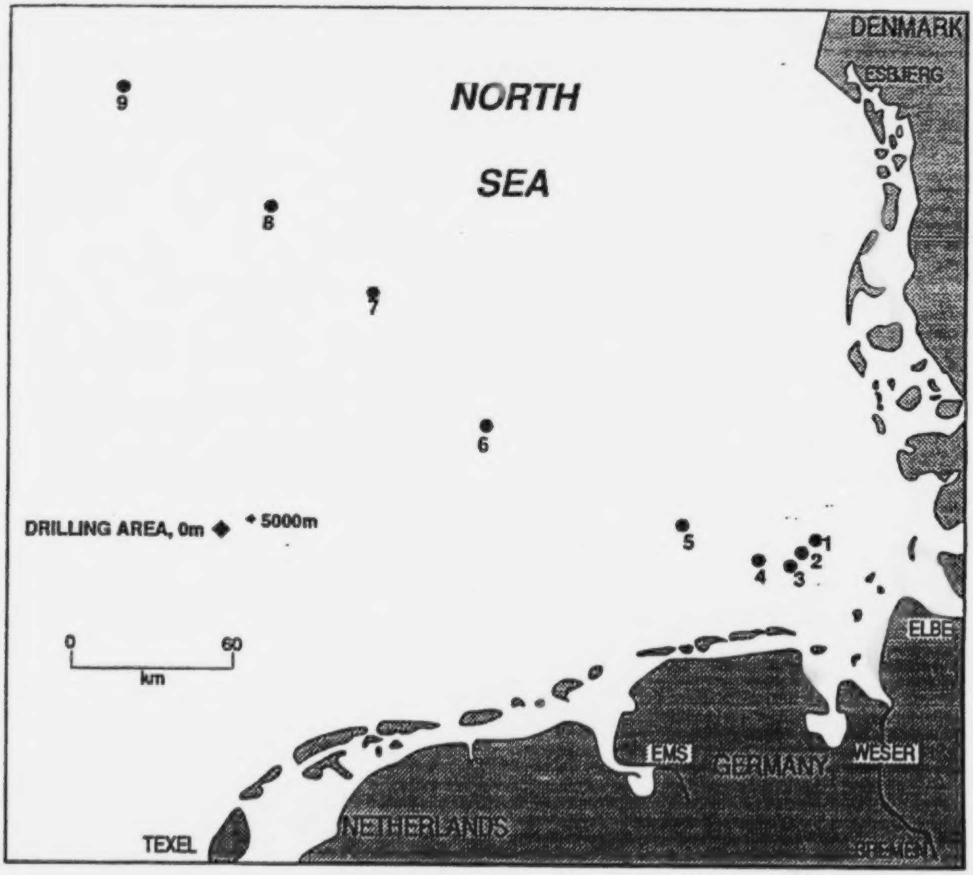
This paper uses data and the results of other workshop studies to determine the pollution status of the tested sediments. Pollution is defined as chemical contamination associated with toxicity and in situ alteration of resident communities. Extrapolations are made to the other major aspects of the workshop (water quality [water column and microlayer chemistry and toxicity], and dab [*Limanda limanda*] pathology).

SEDIMENT CHEMISTRY

Sediment chemical contamination was determined for three basic groups of chemicals: metals, polyaromatic hydrocarbons (PAH) and PCBs (Cofino, In Press). Analyses did not include all low and high molecular weight PAH of possible toxicological concern (e.g., LPAH: acenaphthylene, acenaphthene, fluorene; HPAH: indeno(1,2,3-cd)pyrene; dibenzo(a,h)anthracene; benzo(g,h,i)perylene). However, the PAH were reasonably well characterized as a sufficient number of potentially co-varying PAH were analyzed. More significant omissions include ammonia (Ankley et al., 1990), chlorobenzenes, pesticides, methylated and chlorinated phenols, and alcohols (Chapman et al., 1982).

Sediment chemistry data for contaminants measured along both transects were converted to Ratio-to-Reference values (RTR: Chapman et al., 1987a; Chapman, 1990) to provide clarity in comparisons and data presentation (Tables 1-2). PCBs were only measured along the German Bight transect; trends there followed those of the other measured contaminants (Cofino, in press). Individually measured parameters (chemical contaminant concentrations [Cofino, in press], percent sand and loss on ignition [Chapman et al., in press]) were divided by reference station values for those same parameters. Reference stations (GB09 for the German Bight transect and DA5000m for the Drilling Area transect) were farthest from sources, had lowest overall chemical contaminant concentrations, were not toxic in laboratory bioassays (Chapman et al., in press) and did not contain altered benthic infaunal communities which could have been due to chemical contamination (Kroncke and Rachor, in press; Kroncke et al., in press).

Summarized chemical contaminant data (Tables 1-2) and sediment physical characteristics are compared in Figure 2, as RTR values. Means were determined assuming that values less than detection limits were equal to those detection limits. The Drilling Area transect had highly uniform sediments, and generally similar concentrations of contaminants: metals and LPAH concentrations only varied within a factor of 2; HPAH concentrations varied within a factor of 3.5. Total HC concentrations were more variable, up to a factor of


DENMARK
ESBJERG
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DRILLING AREA, 0m
5000m
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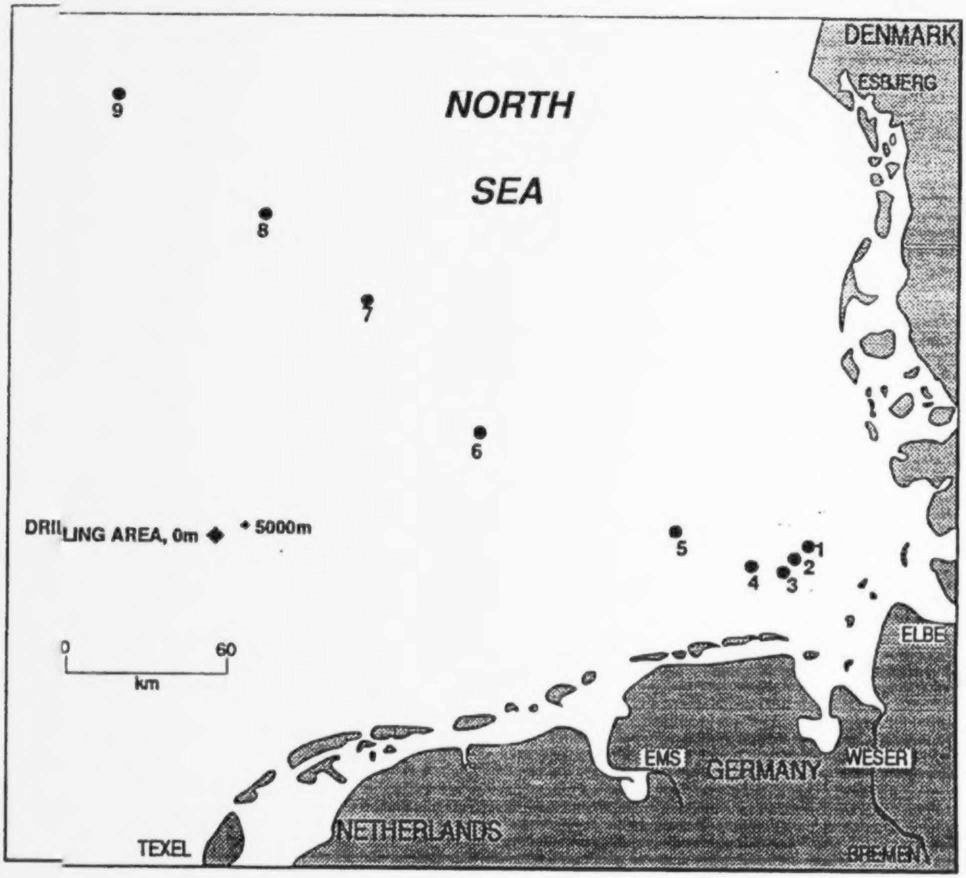
km
ELBE
EMS
GERMANY
WESER
NETHERLANDS
TEXEL
BREMEN

Figure 1. Location of sampling stations. Stations 1-9 are German Bight (GB) stations. Drilling Area (DA) stations are 0; 125; 250; 500; 1,000; 2,000 and 5,000m from the center of drilling.


DENMARK
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DRILLING AREA, 0m
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Figure 1. Location of sampling stations. Stations 1-9 are German Bight (GB) stations. Drilling Area (DA) stations are 0; 125; 250; 500; 1,000; 2,000 and 5,000m from the center of drilling.

Table 1. Ratio-to-Reference (RTR) values for the Drilling Area. Outermost station (5,000 m) used as reference; mean (n = 3) total sediment concentrations used in calculation except for polyaromatic hydrocarbons, PAH (n = 1), see text.

	Distance from Platform (m)						
	0	125	250	500	1,000	2,500	5,000
Metals							
As	1.4	1.2	0.8	0.9	1.1	0.9	1.0
Cd	1.4	1.0	0.9	0.9	0.4	0.9	1.0
Cu	1.4	5.1	1.0	1.0	0.5	0.9	1.0
Hg	1.3	1.2	0.5	0.9	0.5	0.8	1.0
Pb	1.0	1.1	1.1	0.9	0.5	0.9	1.0
Zn	0.9	1.0	0.8	0.9	0.7	0.9	1.0
Mean	1.2	1.8	1.0	0.9	0.6	0.9	1.0
Total Hydrocarbons (HC)							
by UVF	1.4	1.4	1.2	1.1	0.6	1.0	1.0
by GC	45.2	3.2	5.5	40.2	1.2	0.5	1.0
Mean	23.3	2.3	3.4	20.6	0.9	0.8	1.0
Low Mol. Wt. PAH (LPAH)							
Naphthalene	0.5	1.0	0.8	0.9	0.6	0.8	1.0
Methyl naphthalene	<0.2	1.1	<0.2	1.0	0.6	1.5	1.0
Dimethyl naphthalene	<0.1	1.4	0.9	1.4	0.6	<0.1	1.0
Trimethyl naphthalene	<0.1	1.6	1.0	1.2	0.5	1.6	1.0

	Distance from Platform (m)						
	0	125	250	500	1,000	2,500	5,000
Phenanthrene	0.8	0.9	0.7	1.0	0.4	0.8	1.0
Methyl phenanthrene	0.8	0.9	0.9	1.2	0.5	0.9	1.0
Anthracene	1.1	1.1	0.9	1.6	0.5	1.1	1.0
Mean	<0.5	1.1	0.8	1.2	0.5	<1.0	1.0
High Mol. Wt. PAH (HPAH)							
Fluoranthene	<5.4	<7.6	<5.4	≤8.6	<3.2	<7.6	1.0
Pyrene	<4.8	<6.6	<4.8	≤7.0	<2.6	<5.8	1.0
Benz(a)anthracene	0.2	0.2	0.2	0.2	<0.1	0.2	1.0
Chrysene + triphenylene	0.1	0.2	0.1	0.2	0.1	0.1	1.0
Benzofluoranthenes	<2.4	<1.0	<6.0	<6.4	<3.6	≤6.6	1.0
Benzo(e)pyrene	<1.1	<1.0	<1.6	≤2.4	<1.5	<1.9	1.0
Benzo(a)pyrene	<1.0	<1.0	<1.0	≤1.4	<1.0	<1.0	1.0
Perylene	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.0
Mean	<2.0	<2.3	<2.5	≤3.4	<1.6	<3.0	1.0

Underline = highest RTR value

Table 2. Ratio-to-Reference (RTR) values for the German Bight. Outermost station (GB09) used as reference; mean (n = 3) total sediment concentrations used in calculation except for polyaromatic hydrocarbons, PAH (n = 1), see text.

	Stations → Increasing Distance from Elbe River								
	GB01	GB02	GB03	GB04	GB05	GB06	GB07	GB08	GB09
Metals									
As	14.2	7.4	8.6	5.0	4.6	4.2	2.8	3.8	1.0
Cd	222.7	91.7	100.3	44.0	40.7	5.7	1.3	1.0	1.0
Cu	12.4	4.5	5.7	2.6	4.0	1.9	1.5	2.1	1.0
Hg	73.0	17.0	20.5	7.5	4.5	2.3	1.0	1.0	1.0
Pb	13.4	5.4	6.4	3.7	3.2	2.5	1.9	1.4	1.0
Zn	42.7	45.3	32.7	22.9	17.5	10.7	7.1	3.4	1.0
Mean	63.1	28.6	29.0	14.3	12.4	4.6	2.6	2.1	1.0
Total Hydrocarbons (HC)									
by UVF	52.7	20.6	25.0	15.2	17.1	19.6	11.2	3.4	1.0
by GC	51.0	114.0	4.5	0.5	5.5	14.0	4.5	3.5	1.0
Mean	51.8	67.3	14.8	7.8	11.3	16.8	7.8	3.4	1.0
Low Mol. Wt. PAH (LPAH)									
Naphthalene	≤86	<25	<33	<14	<6.5	<14	<5	<1.6	1.0
Methyl naphthalene	≤24	<7.8	<11.6	<5.4	<3.6	<10.4	<4.0	<1.2	1.0
Dimethyl naphthalene	≤34	<11	<12	<7.7	<5.7	<13	<6.5	<2	1.0
Trimethyl naphthalene	<22	<6.5	<8.2	<5.5	<5.0	<7.4	<3.0	<1.0	1.0
Phenanthrene	117	27.5	37.5	17.5	14.2	20.8	6.6	3.1	1.0

	Stations → Increasing Distance from Elbe River								
	GB01	GB02	GB03	GB04	GB05	GB06	GB07	GB08	GB09
Methyl phenanthrene	<15.4	<4.2	<5.2	<3.4	<3.2	<5	<1.7	<1.0	1.0
Anthracene	<41	<8.8	<9.9	<4.7	<3.3	<4.4	<1.0	<1.0	1.0
Mean	<48.5	<13.1	<16.8	<8.3	<5.9	<10.7	<4.0	<1.6	1.0
High Mol. Wt. PAH (HPAH)									
Fluoranthene	<140	<55	<73	<27	<25	<30	<8.3	<3.0	1.0
Pyrene	<110	<42	<50	<24	<20	<29	<8.0	<2.5	1.0
Benz(a)anthracene	<36	<12	<18	<7.7	<6.6	<7.9	<2.5	<1.0	1.0
Chrysene + triphenylene	<54	<89	<26	<11	<10	<13	<4.2	<1.4	1.0
Benzofluoranthenes	<92	<27	<40	<22	<18	<25	<11	<1	1.0
Benzo(e)pyrene	<31	<5.9	<17	<8.8	<6.1	<10	<2.9	<1	1.0
Benzo(a)pyrene	<18	<4.1	<9.0	<4.7	<2.8	<7.1	<1.2	<1	1.0
Perylene	<49	<12	<17	<6.8	<4	<3	<1	<1	1.0
Mean	<66.2	<30.9	<31.2	<14	<11.6	<15.6	<4.9	<1.5	1.0

Underline = highest RTR value

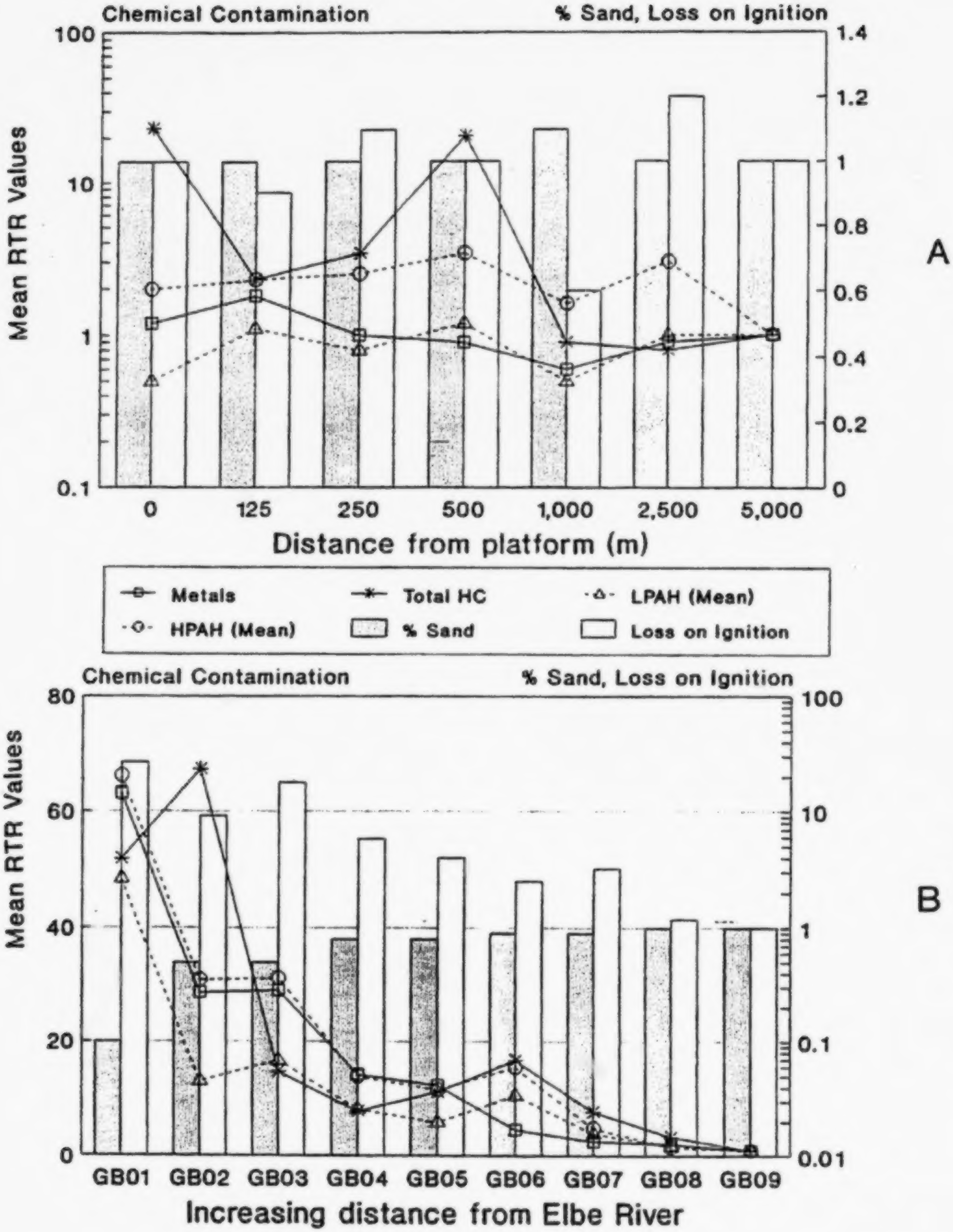

Figure 2. Sediment chemical contaminant data and sediment composition for A = Drilling Area, and B = German Bight. RTR = Ratio to Reference; outermost station (5,000m and GB09) of each gradient used as reference; for explanation, see text.

23.3. This variability in HC was attributable to GC analyses which were much more variable than UVF analyses, and showed no clear trend; very high concentrations were noted at 0 and 500 m with much lower concentrations at all other stations. Oil concentrations in sediments in the center of the Drilling Area were an order of magnitude lower than was measured in 1988, one year after cessation of drilling (de Jong et al., in press).

The German Bight transect, in contrast, had marked differences in sediment characteristics and contaminant concentrations (Figure 2). Highest concentrations of contaminants and finest sediments were found at GB01; contaminant concentrations decreased seaward as did the sediment fines and organic carbon content. Contaminant concentrations were on the order of 50X greater at GB01 than at GB09.

In order to put the measured contaminant concentrations into perspective, highest concentrations of individual chemicals (at GB01) are compared to available sediment quality values in Table 3. This comparison indicates that none of the sediments would be expected to be highly toxic (as was, in fact, determined by actual testing). Even the highest measured chemical concentrations rarely exceeded the sediment quality values, and then only at lower effects levels. Further, except for mercury, exceedances of sediment quality values only ranged from 1.1 to 1.8X; mercury exceedances were 2.9X. Because sediment quality values are useful primarily for screening purposes (Chapman, 1989) and divergence is as common as consensus among different approaches (Long, 1989b), exceedances coupled with measured effects do not necessarily indicate cause and effect. Although future research at the inner German Bight stations could usefully focus on chemicals such as mercury, lead and dimethylnaphthalene which exceed more than one sediment quality value, not all chemicals which could have caused observed toxicity were measured.

SEDIMENT TOXICITY

Sediment toxicity results have been summarized by Chapman et al. (in press b). Trends in toxicity are further summarized in Tables 4 and 5. Complete agreement between different toxicity tests only occurs when sediments are either highly toxic, or non-toxic (Chapman, 1986b, 1988, 1989). The Drilling Area and German Bight sediments both had intermediate, low toxicity, hence the observed differences between test results is expected.

Overall, there was a general trend of higher toxicity closest to sources on both transects (DA0m and GB01). This trend is best seen in primary toxicity test end-points, which are routinely accorded more weight than secondary end-points (Chapman, 1988). Thain (in press) demonstrated a similar trend along the German Bight transect based on oyster larvae 24-h sediment elutriate toxicity tests conducted on shipboard during a cruise one week earlier than the other tests (Butler et al., in press). Some of the toxicity tests which did not follow this trend experienced difficulties in meeting quality assurance/quality control (QA/QC) requirements (Chapman et al., in press); all tests showing this trend met these requirements.

INFAUNAL BENTHIC COMMUNITY STRUCTURE

Descriptions and interpretative analyses of the benthic macrofaunal communities along the German Bight and Drilling Area transects are provided by Kroncke and Rachor (in press) and Kroncke et al. (in press), respectively. In the German Bight, differences in sediment type (e.g., Figure 2) "... hide possible changes along the gradient due to pollution or eutrophication." (Kroncke and Rachor, in press). However, stations closest to the Elbe River had the lowest number of individuals and species (least at Station GB01); increasing numbers of species, individuals and diversity were found offshore. Although Station GB09 benthic infaunal community structure was different than all other stations, this difference was attributed to location and sediment physical characteristics rather than to toxic chemicals. In contrast, Stations GB01, 02 and 03 "...seem to have reached the polluted or grossly polluted state, where only a few resistant species are present." (Kroncke and Rachor, in press).

In the Drilling Area, sediments had basically the same grain-size and organic carbon content (cf. Figure 2); any differences between benthic communities at these stations could not have been attributed to these abiotic factors. Although decreasing trends in species number, biomass and total abundance, particularly of deep burrowers, were noted within 1000 m of the platform and the 0 m station was clearly separated from all other stations based on cluster analysis, Kroncke et al. (in press) found no significant differences between stations. Rather, available evidence indicated that the fauna had recovered from what had previously been readily discernable discharge-related community-level changes (Daan et al., 1990; de Jong et al., 1991, in press). Discharge ceased three years previous to this workshop and subsequent storm action had covered the original sediments containing oil and other discharges with a layer of clean material at the time of sampling (Kroncke et al., in press).

Benthic infaunal communities along both transects showed no clear evidence of major pollution-related impacts. Although benthic communities nearest to each of the two potential sources showed changes that could have been due to toxic contamination, the evidence for such changes did not derive from all methods of data analysis and interpretation. For instance, ABC curves indicated that all Drilling Area stations "...form part of an undisturbed community", while multivariate analyses indicated "...that there is still a gradient in the macrofauna community structure similar to the one found shortly after the discharge had taken place" (Kroncke et al., in press). Thus, the benthic macrofauna results suggested changes close to sources but were far from definitive.

Table 3. Comparison of sediment contamination data with relevant, available sediment quality values

Contaminants	Highest Concentration (Station)		1988 AET[a]			PSDDA Screening Levels[b]	Dutch Soil Pollution Clean-Up Guidelines, Level A[c]	Long and Morgan (1990) Apparent Effects Thresholds[d]		WDOE (1991) Sediment Management Standards[e]	
			Amphipod	Oyster Larvae	Benthos			Low	Median	Criteria	Clean-Up
METALS (µg/g dry wt.)											
As	7.1	(GB01)	93	700	57	57	20	33	85	57	93
Cd	0.668	(GB01)	6.7	9.6	5.1	0.96	1.0	5	9	5.1	6.7
Cu	17.3	(GB01)	1300	390	530	81	50	70	390	390	390
Hg	0.438	(GB01)	2.1	0.6	2.1	0.21	0.5	0.15	1.3	0.41	0.59
Pb	53.6	(GB01)	660	660	450	66	50	35	110	450	530
Zn	104.3	(GB02)	960	1600	410	160	200	120	270	410	960
ORGANICS (µg/kg dry wt.)										mg/Kg OC[f]	
Total PCBs			3100	1100	1000	130	50	50	400	12	65
LPAH											
Naphthalene	86	(GB01)	2400	2100	2700	210	100	340	2100	99	170
Dimethyl-naphthalene	120	(GB01)	1900	670	1400	67	NA	65	670	38	64
Phenanthrene	140	(GB01)	6900	1500	5400	320	100	225	1380	100	480
Anthracene	41	(GB01)	13000	960	4400	130	100	85	960	220	1200
HPAH											
Fluoranthene	140	(GB01)	30000	2500	24000	630	100	600	3600	160	1200
Pyrene	110	(GB01)	16000	3300	16000	430	100	350	2200	1000	1400
Benz(a)anthracene	36	(GB01)	5100	1600	5100	450	NA	230	1600	110	270
Benzofluoranthenes	92	(GB01)	7800	3600	9900	800	NA	NA	NA	230	450
Benzo(a)pyrene	18	(GB01)	3000	1600	3600	680	50	400	2500	99	210

Table 3. (Continued)

[a] AET = Apparent Effects Threshold, defined as sediment concentration above which statistically significant ($P \leq 0.05$) biological effects always occur and, therefore, are always expected (PTI Environmental Services, Inc., 1988).

[b] PSDDA = Puget Sound Dredge Disposal Analysis; screening levels based on AETs, defined as sediment concentration below which biological effects will not occur and should not be expected (PSDDA, 1989).

[c] Concentrations below "A" levels are considered indicative of unpolluted conditions and are calculated background levels in Dutch soil or, for PAH, the detection limits (Netherlands, 1983). These guidelines are not based on demonstrated adverse biological effects nor are they normally applied to in-place marine sediments; however, they are below warning levels for freshwater sediments proposed by the Dutch Ministry of Transport and Public Works (1989).

[d] Concentrations derived based on exhaustive review of chemical concentrations observed or predicted to be associated with biological effects (including AET and PSDDA Screening levels). Low = lower 10% of the data; Median = median of the data.

[e] Values below criteria are designated as having no adverse effects on biological resources; values above clean-up levels indicate that sites should be considered for clean-up; these standards only apply to Puget Sound sediments.

[f] OC - Organic Carbon; to normalize to OC, divide the dry weight by the decimal fraction representing % TOC (Total OC) content of the sediment. None of these values are below measured values normalized to organic carbon.

Underline = Sediment quality value below highest measured concentration.
NA = Not Applicable, no values.

Table 4. Trends in sediment toxicity tests (Chapman et al., in press).

Investigators	Country	Test Organism	End-Points (* = primary)	Results: Trend of higher toxicity*	
				at DA near platform	at GB near Elbe R.
Swartz	USA	*Rhepoxynius abronius* (amphipod)	Survival*	yes	yes
			Reburial	no trend	no trend
Chapman	Canada	*Rhepoxynius abronius* (amphipod)	Survival*	yes	yes
			Avoidance	no trend	no trend
			Reburial	no trend	no trend
		Neanthes arenaceodentata (polychaete)	Survival	no trend	perhaps
			Growth*	no trend	perhaps
		Crassostrea gigas (oyster)	Survival	yes	yes
			Development*	yes	yes
Roddie/Butler	UK	*Corophium 2olutator* (amphipod)	Survival*	yes	perhaps
			Immobilisation	no trend	no trend
		Crassostrea gigas (oyster)	Survival*	yes	perhaps
			Development	yes	perhaps
		Microtox (bacteria)	Bioluminescence *	yes	yes
Phelps	USA	*Mya arenaria* (clam)	Burrowing*	no trend	no trend
		Crassostrea gigas (oyster)	Survival	no; reverse trend	yes
			Metamorphosis*	no; reverse trend	yes
van den Hurk	Holland	*Crassostrea gigas* (oyster)	Survival*	no; reverse trend	no; bell-shaped curve
		Bathyporeia sarsi (amphipod)	Survival*	yes	yes
			Reburial	yes	no trend

* yes = clear trend; perhaps = trend uncertain; no trend = all stations similar; reverse/different trend = clear opposing trend.

Table 5. Summarized sediment toxicity test trends.

Trends:	Number of Responses (%)			
	at DA near platform		at GB near Elbe River	
	Primary end-points	Secondary end-points	Primary end-points	Secondary end-points
Clear trend; higher toxicity	7 (64%)	3 (33%)	5 (46%)	2 (22%)
Trend of higher toxicity possible, but not certain	0 (0%)	0 (0%)	3 (27%)	2 (22%)
No trend; all stations similar	2 (18%)	5 (56%)	2 (18%)	5 (56%)
Clear trend; no higher toxicity	2 (18%)	1 (11%)	1 (9%)	0 (0%)
	11 (100%)	9 (100%)	11 (100%)	9 (100%)

INTEGRATIVE ASSESSMENT

Integrative assessments, reviewed by Chapman et al. (1992) are defined as investigations involving attempts to integrate measures of environmental quality to make an overall assessment of the status of the system. Such assessments can involve two or more of the following components: sediment toxicity tests, sediment chemical analyses, tissue chemical analyses, pathological studies, community structure studies. The present workshop included all five possible components, with particular emphasis on the Sediment Quality Triad (Chapman, 1990).

Analyses of Triad data can involve comparisons of RTR values, ranking, and multivariate analyses, in particular Mantel's test (Mantel, 1967; Legendre and Fortin, 1989). Full details of various methods of Triad data analyses are provided in Chapman (1992). Complex methods of data analyses, derived primarily for situations where contamination and toxicity derive from a variety of sources, were not necessary in the present study where two transects were studied, beginning at clear point sources. Information provided by comparing sediment toxicity, chemistry and benthic infauna alteration, as discussed above, is summarized in Table 6.

Pollution-induced degradation (defined as positive responses to all three Triad components resulting in an adverse change to resident communities including bottom fish and benthic infauna) is only clearly demonstrated at the nearshore GB stations and was moderate rather than extreme. Low levels of pollution and degradation may also be occurring in the center of the Drilling Area, but this is much less certain. All other Drilling Area stations and the outermost six German Bight stations show no evidence of pollution-induced degradation.

Bottomfish information for the two areas focused on dab, *Limanda limanda*. Preneoplastic liver lesions and epidermal ulceration in dab were found, not only in nearshore fish but also, by some measures, offshore over the Dogger Bank. The frequency of abnormalities and malformations in fish embryos and larvae in the plankton was elevated in nearshore waters, then declined offshore but increased again over Dogger Bank (Vethaak, in press; Bucke, in press; Hardy, in press; Cameron, in press).

Table 6. Summary of information provided by the Sediment Quality Triad.

Station (s)	Chemical Contamination	Laboratory Toxicity	Benthos Alteration	Pollution-Induced Degradation
GB01 to GB03	+	+	+	· YES · moderate · decreases GB01-03
DA 0m	-	+	+?	· possible, low · effects due to unmeasured chemicals
GB09	-	-	+	· NO · alteration not due to toxic chemicals
GB04 to GB08 DA 125m to DA 5000m	-	-	-	· NO

Responses are shown as either positive (+) or negative (-), indicating whether or not measurable differences from reference conditions were determined.

STATUS OF NORTH SEA SEDIMENTS

Previous studies of the effects of drilling platforms in the North Sea (Gray et al., 1990) have shown a gradient of potential effects (as determined solely based on benthic community structure). Similar gradients around drilling platforms occur in other parts of the world (e.g., the Gulf of Mexico - Chapman et al., 1991b). The Drilling Area gradient only showed possible effects at the original source; previous studies (de Jong et al., 1991, in press) along this gradient have shown contamination, toxicity and benthos alteration (a Triad indicating pollution-induced degradation) extending in 1988 for 500 to 750 m from the center of drilling. Drilling was exploratory and ceased in 1987. The German Bight gradient showed clear effects close inshore, but a similarly clear decrease offshore.

Sediment contamination and toxicity resulting in pollution can be due to both new and historical contamination. Historical contamination is subject, without new inputs, to natural capping with clean sediments due to storm action, as discussed previously. For instance, Mair et al. (1987) and de Jong et al. (1991) have shown recovery of the macrobenthos in the North Sea following termination of drill-cuttings discharges. The speed and extent of recovery will depend on the extent of effects, which is greatest the longer the source has been operating. In the case of oil platforms, the extent of effects can be up to 1000 m (Mair et al., 1987; de Jong et al., 1991).

The present study was not comprehensive in that not all areas of the North Sea were studied. However, it is the largest international exercise to date studying the North Sea and lends itself to certain testable hypotheses. Specifically, based on the results of this study it appears that:

- sediment pollution is not general in the North Sea area (offshore sediments away from sources are not polluted nor degraded although major depositional areas such as the Norwegian Trench [Becker, in press], which have not been fully investigated, could be polluted)
- sediment pollution is generally restricted to the vicinity of point source discharges
    - in the case of oil platforms, on the order of hundreds of meters
    - in the case of major rivers, on the order of tens of kilometres
- cessation of point-source discharges in offshore areas should result in decreased sediment pollution through natural capping (e.g., storm action - sediments are very mobile; Becker, in press).

In addition, there are certain unresolved issues that require further, but focused study. Dogger Bank sediments are clearly not polluted, yet dab collected from the Dogger Bank show evidence of stress. Although the Dogger Bank area comprises a coastal front and such fronts have been shown to accumulate contaminants (Tanabe et al., 1991), there is no evidence of such accumulation in Dogger Bank sediments, which are coarse-grained and hence not depositional. Clearly this stress cannot be due to sediment contamination. Water column tests conducted during this international study showed no evidence for wide-spread contamination and toxicity other than in the surface micro-layer (Hardy, in press; Thain, in press). Dab eggs and embryos are found primarily near the surface of the water column (Rijnsdorp, in press; Cameron, in press). Accordingly, the most likely potential sources of this stress, which merit further research, are:

- surface microlayer (aerial transport of toxicants resulting in contaminated, toxic waters into which buoyant dab eggs float and where exposure occurs resulting in effects realized in older life-stages)
- dab immigration from other areas closer to sources, such that the dab populations at Dogger Bank are not resident (cf. Rijnsdorp, in press).

ACKNOWLEDGMENTS

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DIRECT SEDIMENT TOXICITY TESTING PROCEDURE (DSTTP).

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A qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) using the Toxi-Chromotest kit was developed. This DSTTP has advantages over many other toxicity bioassays in that it is simple, quick, and inexpensive. It does not require any instrumentation and can be easily applied under field conditions. The DSTTP measures the available toxicants of the test sediments, suspended sediments, soil and any other solid wastes without altering the original characteristics of the samples as occurs in extraction and concentration procedures.

THE EFFECT OF HOMEOTHERMY ON BIOMAGNIFICATION.

M.D. Paine, EVS Consultants, North Vancouver, BC, (604) 986-4331.

Fate models usually focus on the transfer of contaminant mass through food chains. Concentration in tissues is contaminant mass/body mass, and the transfer of body mass should also be considered. Thomann (1981) showed that *F*, the concentration of a contaminant in a predator divided by that in its prey, should be ≈a/*E* (a = assimilation rate of contaminant; *E* = energy conversion efficiency of prey to predator mass), if depuration rates and contaminant uptake from solution are negligible. Given that *E* ≈ 0.20 and is independent of body size for poikilotherms, and that *a* ≈ 0.2 - 0.6 for most contaminants with log *Kow* = 4-7, values for *F* are expected to be 1-3. For homeotherms, *E* ≈ 0.02 because of the energy expended on thermoregulation, and *F* should be 10X greater than for poikilotherms. Thus, biomagnification may depend as much or more on the properties of the organisms *E* as on the properties of the contaminant (*a*). Existing data show that *F* in aquatic food chains from prey to poikilotherm predators ranges from < 1-3, as predicted. Support for increased biomagnification for homeotherm predators is more equivocal as values of *F* range from < 1-100, and data are sparse.

AQUATIC TOXIC CONTAMINANTS DATABASE.

J.D. Hamilton, Winnipeg, MN, (204) 983-5338; B. Glassey, Department of Zoology, University of Manitoba, Winnipeg, MN and W.L. Lockhart, Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, MN.

A database is being developed at the Freshwater Institute in an attempt to develop a coherent picture of the state of knowledge of toxic contaminants in fish and marine mammals in Canada. The database includes information on heavy metals and many of the organic contaminants (PCB, PCDD, PCDF) and pesticides (Dieldrin, DDT, DDE, etc.). Each record includes the contaminant level, tissue analyzed, length, weight and % fat of the organism, geographic coordinates and bibliographic reference.

A secondary database is also being set up to include information on the native subsistence consumption of fish and arctic marine mammals.

The information for both databases comes mainly from published reports as well as some unpublished data.

SOME STATISTICAL METHODS FOR ANALYZING VARIATION IN MORPHOMETRICS AND TOTAL MERCURY OF FISH SPECIES WHEN PARAMETRIC ASSUMPTIONS ARE VIOLATED.

L.Z. Florence, (403) 632-8349; T. Clarke, P. Henry, J. Kirtz and K. Smiley, Alberta Environmental Centre, Vegreville, AB.

Assumptions, like independent and normally distributed data having constant variances, which form the basis of traditional parametric statistical methods, e.g. analysis of variance (ANOVA), cannot always be met. Even when these assumptions are met, sample sizes and the level of variability in a parameter, may result in tests having insufficient power to detect real differences. This paper reports on statistical summaries of published sample data collected from Alberta fish species in rivers and lakes for which size (weight and length) and total mercury content (ppm) were taken. Hypotheses are formed and parametric methods, with nonparametric alternatives where applicable, are presented and discussed.

CHARACTERIZATION OF STATISTICAL PROPERTIES OF LOW-LEVEL GC/MS DATA.

C.I. Johnson, (403) 632-8464 and L.Z. Florence, Alberta Environmental Centre, Vegreville, AB.

Data used in environmental assessments may be generated by different laboratories, after using different methods and protocols. The reliability and precision of low-level data will vary among sources. Methods to characterize and quantify these properties in low-level GC/MS data will be presented. Potential causes of these differences will be discussed.

EXPRESSING THE DOSE-RESPONSE RELATIONSHIP: PRACTICAL ASPECTS.

Martin Samoiloff, BioQuest International, 3-2725 Pembina Highway, Winnipeg, MN, R3T 2H5, (204) 269-7264.

Toxicity data require the establishment of the dose-response relationship. Often LC50 values are used as a convenient shorthand means of expressing this relationship. This has led to the misconception among some users of toxicity data that the LC50 value reflects some intrinsic property of the tested material.

This presentation will focus on the best methods for expressing the dose-response relationship and the overall toxicity of a sample for acute and chronic tests, and will review some of the available protocols for obtaining these values.

GROWTH TYPE TOXICITY TESTS: SOME STATISTICAL PROBLEMS.

Glenn F. Atkinson, Environment Canada, Ottawa, ON, (819) 997-8809.

A number of aquatic toxicity tests are now in use, or under development, which measure growth (*Selenastrum capricornutum*, fathead minnow) or something very like growth (reproduction of *Ceriodaphnia dubia*) rather than lethality. These tests present a number of statistical problems.

In many of these growth type tests the dose-response curve is definitely not monotonic. At very low doses, it is common to find there is an increase in response (relative to the control) followed by a decline, often to zero response at high doses (see Figure 1). This is in contrast to the monotonic decreasing response typically seen in lethality tests. Besides this difference in the form of the dose-response function, the nature of the variables measured means that methods applicable to the lethality tests generally cannot be applied.

In a number of these tests the response variable is often transformed to the percentage growth inhibition, defined as

$$I_i = \left(1 - \frac{R_i}{R_C}\right) * 100$$

where R_i and R_C are the responses at the ith concentration and in the control respectively. While this may appear attractive (and may be useful for data presentation) it is not pleasant from a statistical point of view. Suppose that a test is in the best of all worlds; the response variable R_i is normally distributed with equal variances at all concentrations, but, of course, with different means. Then the derived variable I_i will not be normally distributed and the variances will not be equal.

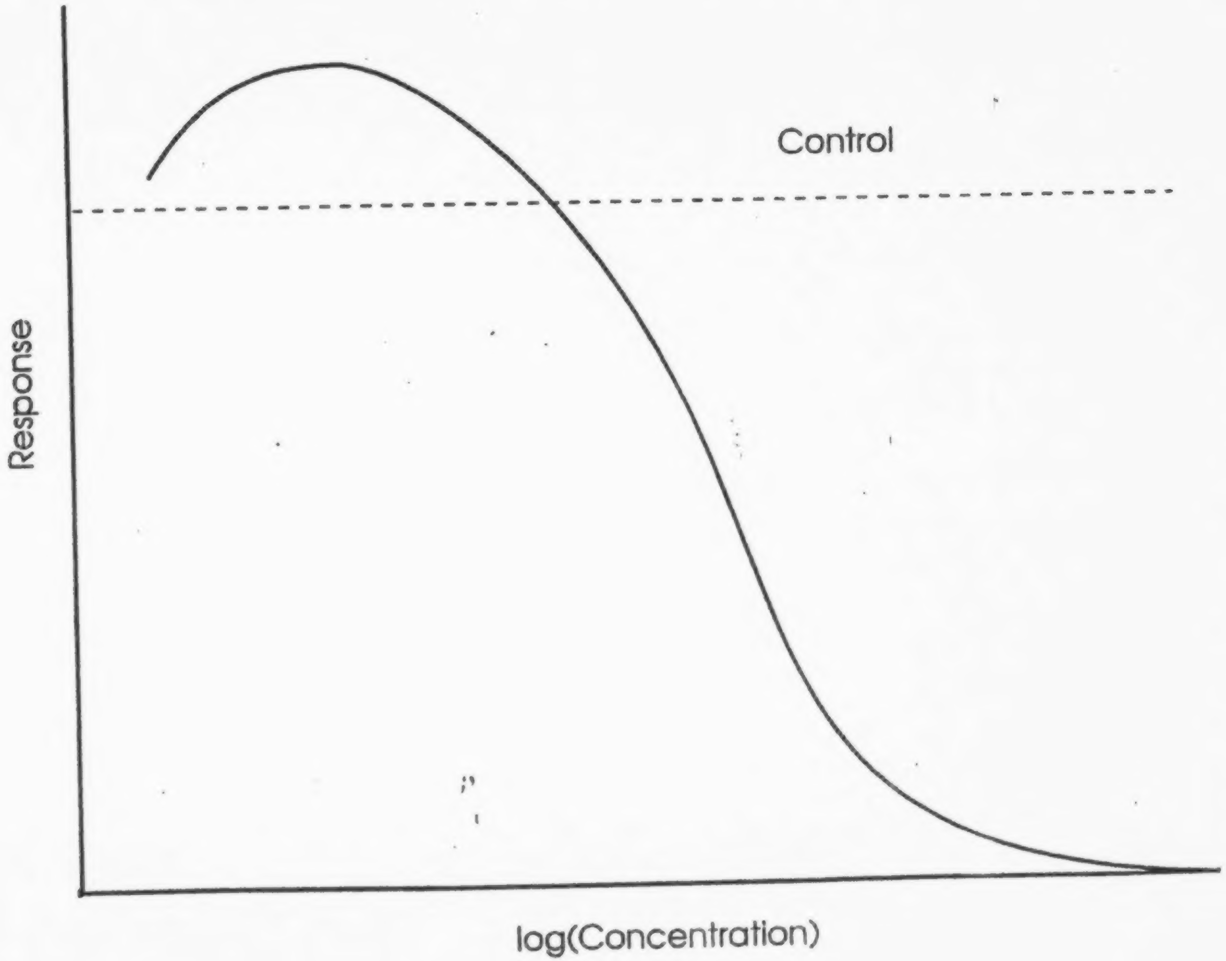
In practice it will make no difference if we look for the concentration for which I is expected to be 25 (say) or for the concentration which would give a response equal to 75% of the control. Expressing the results as a percentage also leads users to think of the variable as similar to a percent lethality, in fact the two variables are quite different. In what follows I will use the response variable (or perhaps a transformation of the response variable) directly.

Test Endpoints

LOEC

The LOEC, the lowest observed effect concentration, is defined as the lowest concentration which is significantly different from the control according to some statistical test. It is important to recognize that the LOEC depends not only on the dose-response function but on the variance of the test and the sample size. Thus if two determinations are made with the same material (hence a single dose-response function) the LOEC can be made smaller by reducing the variation in the test or by increasing the sample size.

Figure 1. Typical dose-response curve for a growth type toxicity test.



The tests commonly recommended for the determination of the LOEC are Williams' test, Dunnett's test, the Bonferroni t-test, and Steel's many to one rank test. These tests will not give equivalent results. In order to examine the operating characteristics of these tests a small simulation study was done. The simulation modelled a test with a control and five dilutions. Random normal variables were generated with means as in the following table and equal variances such that the coefficient of variation of the control was 25%. Four tests were compared: Williams' test, the simple t-test, Dunnett's test, and the Bonferroni t-test. The results, for 1000 simulations, were as follows:

	Expected		Frequency of Decision		
Dose	Response	Williams	t-test	Dunnett	Bonferroni
Control	1				
1	0.90	0.11	0.09	0.02	0.02
2	0.75	0.27	0.27	0.14	0.12
3	0.50	0.46	0.48	0.47	0.46
4	0.20	0.16	0.15	0.32	0.37
5	0	0.01	0.00	0.04	0.05

The average LOEC for the tests was: Williams 2.69, t-test 2.71, Dunnett 3.20, Bonferroni 3.31. The close agreement between Williams and the t-test and between Dunnett and Bonferroni was a little surprising but was maintained in other simulations with different coefficient of variation and different sample size. It is obvious that the tests give different results. What is also of concern is the lack of power; all of the tests give average LOECs about three, a significant difference is found (on the average) for the dose whose expected response is 50% of the control. Is this good enough?

The LOEC appears to be a lineal descendent of what, early in this century, was called the *minimal effective dose*. Trevan in 1927 demonstrated the unsatisfactory nature of this concept:

> The common use of this expression in the literature of the subject would logically involve the assumptions that there is a dose, for any given poison, which is just sufficient to kill all or most of the animals of a given species, and that doses very little smaller would not kill any animals of that species. Any worker, however, accustomed to estimations of toxicity, knows that these assumptions do not represent the truth.

The conclusion is that the LOEC is an unsatisfactory endpoint and should not be used.

ICp

The ICp is the inhibiting concentration for a specified percentage effect. It is a point estimate of the concentration which causes a specified inhibition of effect, relative to a control material. As such it is an estimate of a point on the dose-response curve; for a given test material, and a given control material, it should always be an estimate of the same point. Of course the precision of this estimate will depend on the variation and the sample size of a particular test.

The simplest method of estimating the ICp is by linear interpolation. Find the doses whose response bracket the desired point and do a linear interpolation between these points. An approximate variance for the interpolation estimate can be obtained by treating this estimate as an inverse regression and using the usual formula. While the procedure is simple it uses only a portion of the data from the test, essentially discarding the rest. It may also happen that the portion of the curve is very flat in region of the estimate, if this is the case the variance 'blows up' (see Figure 2).

Note that the estimate of the ICp as implemented in the program BOOTSTRP assumes that the responses form a monotonically decreasing sequence and produces 'adjusted' responses so that this assumption is satisfied. Since, in many cases, the responses do not appear to satisfy this assumption some distortion of the estimates may result.

An alternative is to use a regression estimate; difficulties are encountered because data from these types of tests are almost always obviously non-linear. In some cases linear regression has been used after trimming off part of the data to obtain something near linear. This approach can be dangerous since the 'trimming' is very subjective and can substantially alter the result (see Figure 3). This will be particularly troublesome for small values of inhibition since the curvature generally is most pronounced at the extremes.

For some *Selenastrum* and fathead minnow data reasonable fits were obtained using a quadratic regression of log(cell count) on log(concentration) (see Figure 4). Before recommending this method it will be necessary to examine a greater variety of data to see if this method is generally applicable.

'Active' Diluent

Paraphrasing Wood (1946) the basic assumptions of bioassay are:

a) the response supposed to be produced by the known amounts of 'factor X' is actually due to the factor itself and not some other substance,

b) we assume that the Reference Material contains no substance, other than factor X itself, contributing to the response we measure, and that the Test Material behaves so similarly to the Reference Material that it may be regarded simply as a dilution of the Reference Material in a completely inert diluent.

When testing, for example, effluents we are clearly not in this situation, our Reference Materials are not comparable to the Test Material in the way defined for the classic bioassay. But we need to think about the phrase "dilution ... in a completely inert material". Often in effluent testing the dilution and control water are something like: receiving water, "upstream" water, or surface water. It may be unreasonable to consider this as a "completely inert diluent". What are the consequences? The real world is undoubtably more complex but I will look at a simple model. The assumptions are:

Control water has the same effect as some dilution of the Effluent,
Control water and Effluent have the same mode of action (the same dose-response),
The response is linear on log(concentration).

The essence is that the control water behaves as if it contained some small fraction δ of the active ingredient of the effluent. Now look at the results of using this control water as diluent. If $\delta = 0$ the dose-response is a straight line. If $\delta > 0$ the dose-response is non-linear and depends on the magnitude of δ (see Figure 5). The consequence is that two tests using different controls can obtain quite different results. Is this a desirable situation?

Figure 2. The interpolation estimate and a linear regression estimate when the data is poorly behaved.

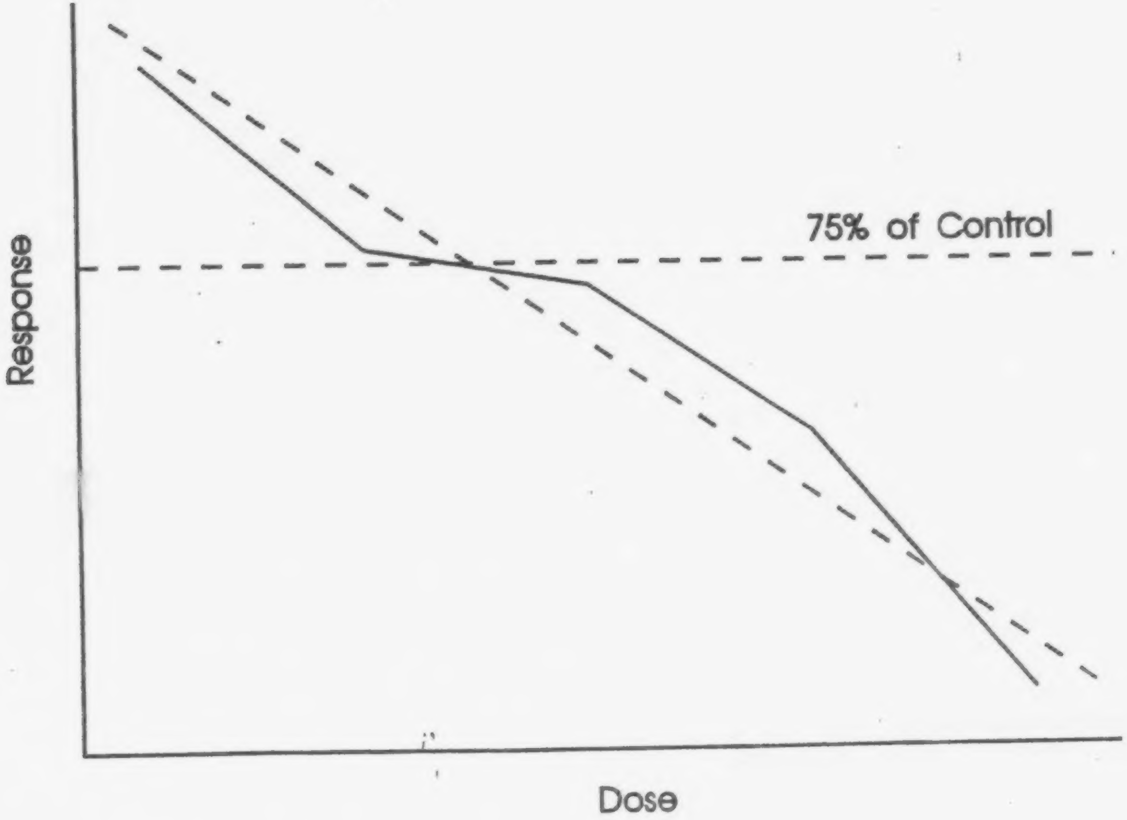


Figure 3. Contrast of linear and curvilinear estimates of the IC75.

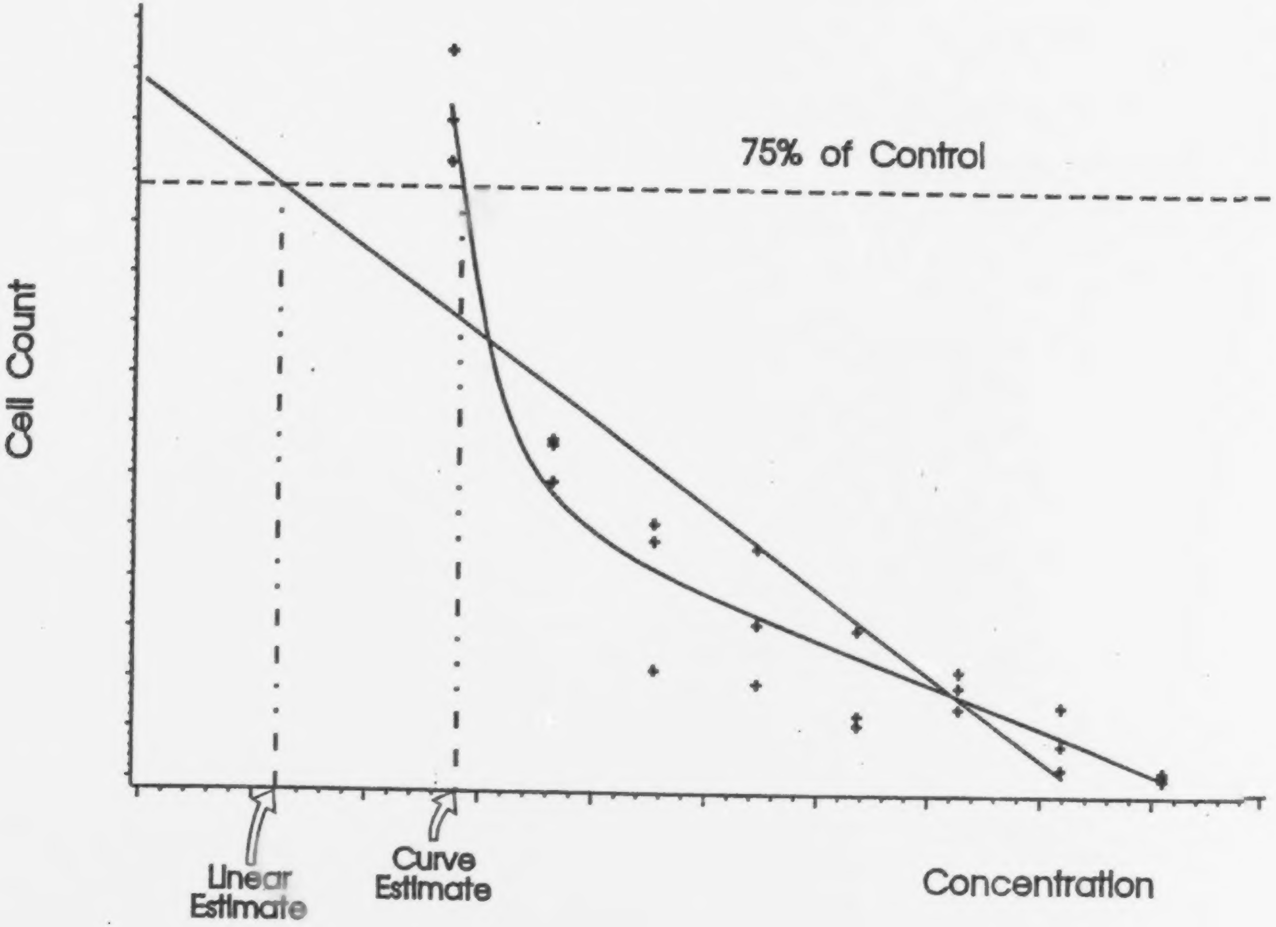


Figure 4. *Selenastrum capricornutum*, a 'nice' example of the fit of a quadratic regression of log (cell count) on log (concentration).

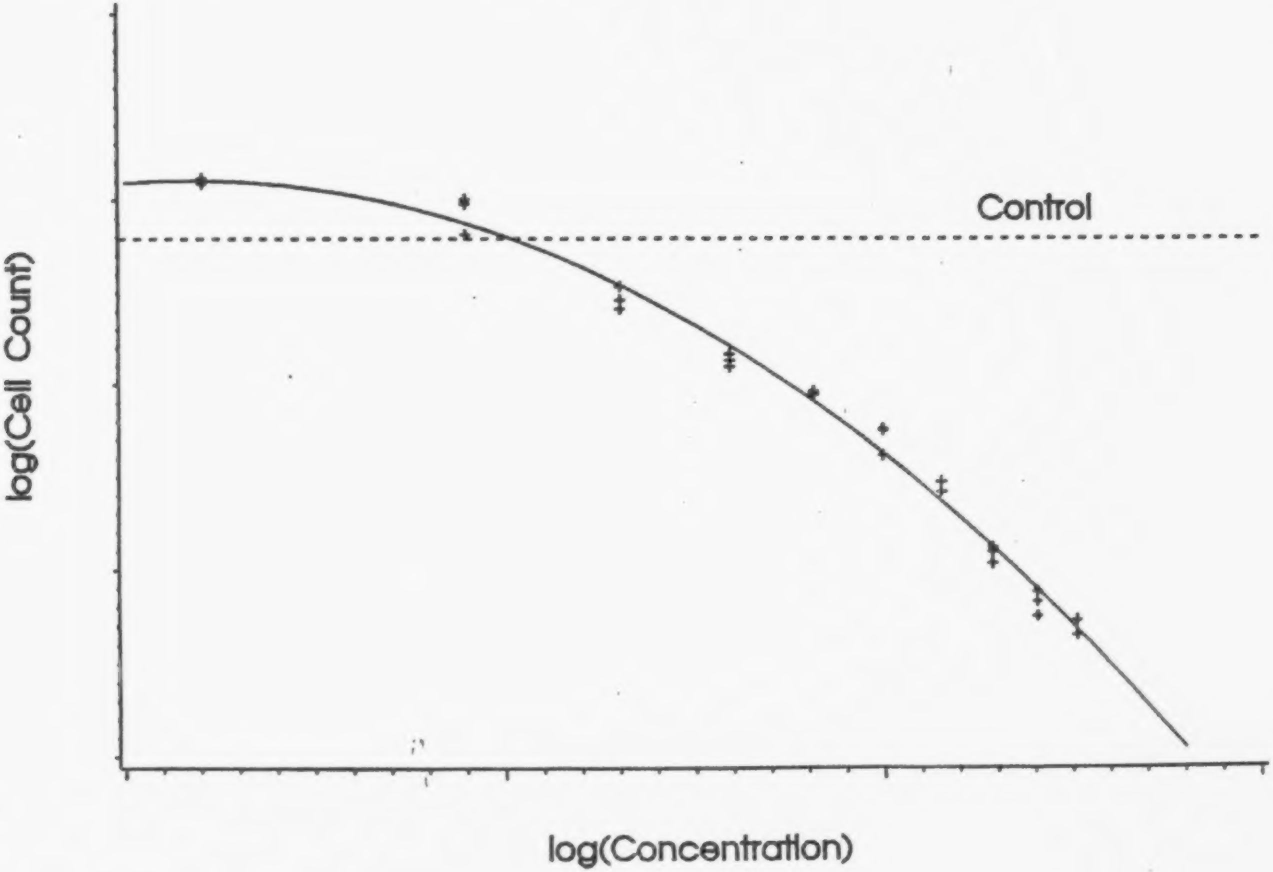
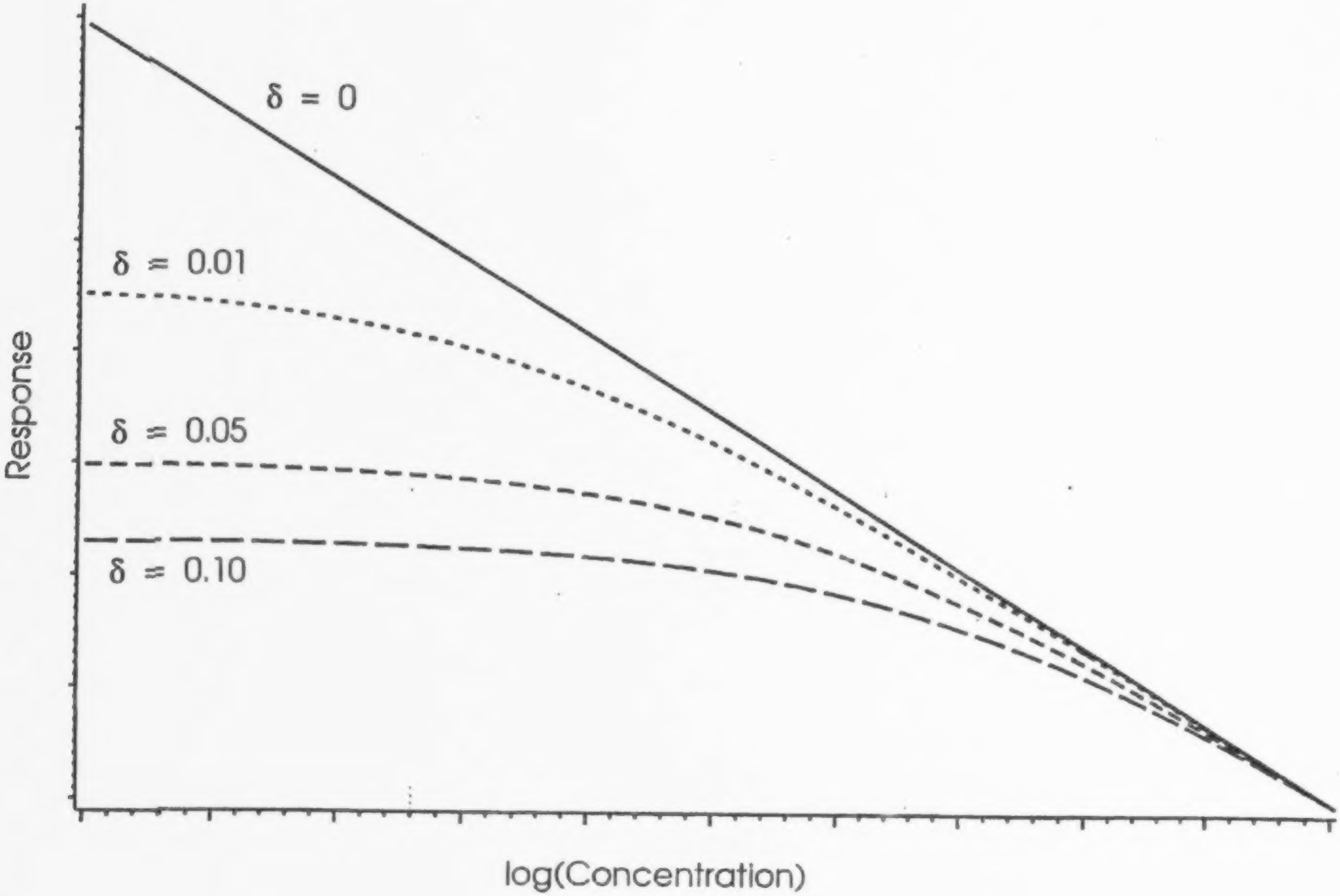


Figure 5. The effect of an 'active' diluent on the dose-response curve.



QA/QC FOR THE SMALL COMMERCIAL BIOASSESSMENT LABORATORY. Martin Samoiloff, BioQuest International, 3-2725 Pembina Highway, Winnipeg, MN, R3T 2H5, (204) 269-7264.

Although the demands for rigorous quality control/quality assurance are as great for small facilities as for large facilities, the process of establishing such practices is often more difficult in smaller facilities. The integration of total quality management into a small laboratory involves three complementary activities:

1. The assignment of specific quality-monitoring roles to all staff members.
2. The focus of quality management at the operational level rather than at the test batch level.
3. The utilization of external evaluators in the quality management process.

These activities must be implemented despite the constraints of limited staff numbers and capital. Methods of implementation will be discussed.

A PROPOSED NATIONAL FRAMEWORK FOR ECOLOGICAL RISK ASSESSMENT AT CONTAMINATED SITES IN CANADA.

C. Gaudet and D. Milne, Eco-Health Branch, Environment Canada, Hull, PQ.

Introduction

In response to a growing public concern that contaminated sites in Canada were placing human health and the environment at risk, the Canadian Council of Ministers of The Environment[2] (CCME) initiated the National Contaminated Sites Remediation Program (NCSRP) in 1989 to promote the remediation (cleanup) of high priority contaminated sites. To ensure national consistency in the assessment and remediation of sites, the CCME requested the development of common scientific tools. Ecological risk assessment was considered to be a critical component in this overall framework for contaminated site assessment and remediation (Gaudet et al. 1991). Accordingly, the CCME requested the development of national guidance in conducting ecological risk assessment at contaminated sites.

Ecological risk assessment is a new and evolving science encompassing fields as diverse as ecotoxicology, population biology, ecology and contaminant fate and behaviour. The inherent complexity of this emerging field, coupled with the inconsistency in existing approaches both nationally and world-wide, presents unique challenges in development of comprehensive national framework for Canada. In developing a national ERA guidance framework for Canada, an extensive review of existing approaches was first conducted (EVS 1992). Based on an evaluation of the strengths and weaknesses of these approaches, several recommendations were made for a Canadian framework.

When is an ERA required?

The ultimate goal of an ERA for contaminated sites is to determine whether or not, and to what level remediation is necessary and in cases where treatment is required, to help specify appropriate remediation targets. Ecological risk assessments can be used to define problems, set priorities, focus investigations, and plan remediation efforts.

To assist decision makers faced with determining whether on not to select an ERA as part of the process of contaminated site assessment and remediation, factors that may trigger an ERA are proposed. It is assumed that the decision will normally be based on a preliminary site characterization, and it is recognized that (1) priorities and available information to support an ERA may vary between different jurisdictions and (2) that local policy and public concern may shift the decision to conduct an ERA.

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[2] The CCME is comprised of federal, provincial and territorial Minister's of the Environment. The CCME National Ecological Risk Assessment Framework is being developed in collaboration with the CCME Subcommittee on Environmental Quality Criteria for Contaminated Sites.

Additional ERA triggers can be grouped into three categories:

- factors that pertain to significant ecological concerns (critical habitat for wildlife; rare or threatened species, populations or ecosystems present; lands designated as a natural area or lands important for fishing, hunting or trapping);
- issues concerning unacceptable data gaps (one or more chemicals about which little is known are present; exposure conditions and hazards levels are uncertain or unpredictable; there are significant gaps in available information concerning ecological receptors);
- additional circumstances (costs of remediation to meet existing environmental criteria are extremely high and/or the contaminated area is very large and priorities must be established).

The need for a national ERA framework

ERAs are highly site-specific and no single, standard design can be expected to apply equally to all contaminated sites in Canada. In many ways, each individual ERA will be unique and require an original, innovative plan of investigation and action. Nevertheless, the basic elements in an ERA can be standardized, within a framework to ensure a comprehensive nationally consistent approach to risk assessment so that each assessment not only provides answers to site-specific management questions, but also meets the NCSRP mandate. Standardization is important because it promotes development of a national program that ensures comparability between regions and facilitates national reviews and interpretation across all sites.

Besides being inconsistent across sites ERAs are often overly complex and open-ended making the investigations difficult to interpret when setting remediation objectives for contaminated sites. An ERA framework can serve as a template for designing and conducting ecological risk assessment at the appropriate level of effort.

A three-tier (three-level) strategy composed of sequentially more sophisticated and complex evaluations is recommended for use in the NCSRP. Sequential evaluation and feedback allow sound scientific judgements and efficient use of resources by minimizing unnecessary data collection so that major effort can be focused in areas with the greatest benefit (Makie and Duthie, 1978).

While the recommended Canadian framework provides a common scientific basis for consistent and effective Ecological Risk Assessment, given the broad differences that will exist between sites in terms of ecological concerns, potential uses of the site, and the types and behaviour of contaminants present, a national guidance framework cannot provide a "recipe book" for ERA. A national approach must be flexible enough to accommodate not only site differences, but the myriad of different and evolving techniques for conducting an ERA such as bioassay, field sampling, and contaminant fate modelling.

The proposed ERA framework

As mentioned, the proposed framework consists of a three tier strategy composed of sequentially more sophisticated and complex investigations. Each level in this tiered approach to ERA has the same four components and include:

- exposure assessment (sources of stressors; magnitude, duration and frequency of exposure);
- receptor characterization (which are the important receptors and habitats?);
- hazard assessment (toxicity of stressors, modifying factors and measurement of responses);
- risk characterization (biological response to dose/concentration; magnitude, significance and probability of effects from the estimated exposure).

These components are investigated and evaluated at each tier. Progress through the tiers is dependant upon the complexity of the contaminated site and can be halted at any tier within the framework if there is sufficient information to arrive at a remediation action plan. Also, it is an option at any level to proceed to the next level of complexity for only one or a few components of the framework. For example, at the end of Level Two, a decision may be made to proceed to Level Three only for exposure and hazard studies if enough is already known about the species to warrant no further study.

Level one is characterized by simple, qualitative and/or comparative methods, and relies heavily on literature information and previously collected data. Level One studies are likely to be focused mainly at the species level and to be descriptive as opposed to predictive. Output from this tier would include a preliminary, quantitative estimate of exposure via the dominant pathway(s), the basic life history information on species identified as potential receptors; LC50, LD50 and/or benchmark concentrations for selected chemicals and species; a qualitative characterization or risk as "high", "intermediate", or "low"; estimates of uncertainty restricted to safety factors and identification of data gaps.

Level two is intermediate between Levels One and Three and provides semi-quantitative information. ERA tools that fit within Level Two include standard environmental methods and models as well as specialized approaches developed for ERA. There is an increased emphasis on data collection and with a focus on priority issues, as determined during Level One investigations. Level Two investigations concentrate on the population and community levels. Expected results from a tier two investigation include: quantitative estimates of Expected Environmental Concentrations (EEC) with estimates of uncertainty; detailed life history data and food web interactions; LC50, LD50 and continuous exposure/response relationships obtained from toxicity testing; statistically analyzed population and/or community data; uncertainty estimates.

Level three relies on site specific data and predictive modelling to supply quantitative information, particularly on complex ecosystem responses. Chronic effects, interaction between chemicals, and ecosystem levels are encompassed in Level Three ERA. This is the level at which a number of the more complex USA EPA procedures, methods and tools operate. While the value of this refined and sophisticated approach is recognized, the resources required may not always be warranted. Again, the output expected from this level of investigation would include; advanced quantitative fate models incorporating the most important pathways of individual chemicals or mixtures; exposure/response relationships for survival, growth and reproduction of all vulnerable ecosystem components; exposure/response relationships for population, community and/or ecosystem; quantified estimates of risk and associated uncertainty.

Future Directions

Validation and demonstration of the proposed framework is to be carried out in the near future through application to case study example(s). In conjunction with this project, a document that will provide practical guidance to site personnel in the design and implementation of an ERA is also being prepared. Both tasks are being carried out by a recently established Ecological Risk Assessment Working Group, which is comprised of federal and provincial environmental personnel.

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CASE STUDIES IN ECOTOXICOLOGICAL ASSESSMENT OF COMPLEX MIXTURES. C. Thellen, L. Martel and R. Chassé, Ministère de l'Environment du Québec, Sainte-Foy, PQ, (418) 646-1301.

The basic aim of this paper is to outline the utility of ecotoxicological assessment in support of environmental management of complex mixtures. The paper will be focused on three studies, with special emphasis on necessary endpoints to generate ecotoxicological hazard and risk assessment, in predictive concern. A first study will focus on integration of literature data and laboratory data to establish ecotoxicological hazard of different forest fire extinguishers. For this, a series of toxicological and fate data based on major components of each product were obtained from literature and compared to assays performed with operational mixture. A second study will insist on use of worst possible case scenarios and models to generate an ecotoxicological risk of a specific fire foam. In this study, hazard was based on laboratory data related to the source, fate and effects, exposure was elaborated from theoretical scenario of dispersion of the product, while risk of effect was developed from a fish population model (RAMAS). A third study will discuss laboratory and field experiments applied to assess risk of dust suppressants. This specific study necessitated a series of field simulation on experimental roads in support to the hazard assessment established from laboratory analyses. These approaches will be discussed on the basis of different descriptive activities in support to a common assessment activity.

As part of decision-making scheme, an ecotoxicological framework relating source-driven and effect-driven assessments will finally be presented.

ISSUES IN ECOTOXICOLOGY AND RISK ASSESSMENT: EXPOSURE CONCENTRATIONS VERSUS CRITICAL BODY RESIDUES. L.S. McCarty, Scientific Research & Consulting, Oakville, ON and D. Mackay, University of Toronto, Toronto, ON.

The increasing use of modelling in toxicity evaluation and in exposure models associated with risk assessment reveal a common problem: what is the toxicological significance of body or tissue(s) residues? This presentation will focus on the current status of residue-based toxicity interpretation for organic chemicals. Two main areas will be examined:

1. body residues associated with different modes of toxic action;
2. body residues for various acute and chronic bioassay endpoints.

The modes of aquatic toxic action identified by McKim, Bradbury and co-workers plus dioxin toxicity are examined for representative critical body residues (CBR). CBR for chronic toxicity endpoints are discussed in terms of Mayer's view of acute/chronic relationships. Supporting information from QSARs and residue literature are provided.

The utility and role of modelled and field-observed body residues in the environmental regulatory process will be discussed.

ECOLOGICAL RISK ASSESSMENT OF AN ECDYSONE AGONIST INSECTICIDE FOR FORESTRY.

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ABSTRACT

RH-5992 is a new ecdysone agonist insecticide which interferes with the normal molting process in larval Lepidoptera. The larvae stop feeding within hours of exposure to RH-5992 and soon undergo an unsuccessful (lethal) molt. Testing has demonstrated that RH-5992 is highly effective against several important forest pests, such as spruce budworm, gypsy moth, hemlock looper, forest tent caterpillar and other Lepidoptera pests in both deciduous and conifer tree crops at rates of 35 to 70 g/ha. An ecological risk assessment addressing both terrestrial and aquatic risks supports the environmentally responsible nature of RH-5992 for forestry uses. Environmental fate and persistence studies support expected exposure levels, and ecological effect studies document maximum acceptable environmental concentrations for mammals, avian species, and aquatic organisms. Exposure scenarios include penetration through the forest canopy onto soil and litter, spray drift, direct overspray onto water bodies and runoff.

EXTENDED SUMMARY

RH-5992 is a new ecdysone agonist insecticide which interferes with the normal molting process in larval Lepidoptera. RH-5992 is the Rohm and Haas Company research code number for tebufenozide (proposed), the active ingredient (a.i.) in RH-5992 technical and the aqueous flowable formulation, RH-5992 2F (MIMIC™, CONFIRM™). The larvae stop feeding within hours of exposure to RH-5992 and soon undergo an unsuccessful (lethal) molt. Testing has demonstrated that RH-5992 is highly effective against several important forest pests, such as spruce budworm, gypsy moth, hemlock looper, forest tent caterpillar and other Lepidoptera pests in both deciduous and conifer tree crops at rates of 35 to 70 g/ha. The compound has shown negligible activity against other nontarget insects such as aphids, beetles and flies.

Tebufenozide [benzoic acid,3,5-dimethyl-1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl) hydrazide (CAS Registry Number 112410-23-8)] has a water solubility of 0.83 mg/L and a negligible vapour pressure. Experimental fate and persistence studies demonstrate low soil and sediment mobility, low runoff potential and low measured bioconcentration in fish. Aerobic soil and sediment half-lives average approximately 100 d in laboratory studies.

Ecotoxicity characterization of RH-5992 supports low nontarget organism toxicity for both acute and chronic exposures and aquatic and terrestrial matrices. Aquatic LC (EC) 50 values range from 3.0 to 5.7 mg ai/L for fish and daphnids and chronic no observed effect concentrations (NOEC) am 0.71 mg ai/L for fathead minnows and 0.029 mg ai/L for daphnids. Acute terrestrial toxicities in avian and mammalian species are greater than 5000 ppm. Chronic terrestrial toxicity studies are in progress. Also, RH-5992 is not considered toxic to honeybees and earthworms.

Exposure scenarios include penetration through the forest canopy onto short range grass (Hoerger & Kenaga 1972), soil and litter, spray drift, direct overspray onto water bodies and runoff. We used USA EPA-accepted ecological risk assessment guidelines (USA EPA 1986, measured and maximum canopy penetration (Barry 1984; Sundaram 1991a; 1992b; Szeto & Sundaram 1981), and RH-5992 specific inputs to complete the ecological risk assessment. The proposed forestry application of RH-5992 does not present unreasonable acute risks to avian or mammalian wildlife, nor unreasonable acute or chronic risks to aquatic organisms and other non target or endangered species. The ecological risk assessment supports RH-5992 as an environmentally responsible forestry insecticide.

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ISOLATION OF TOXIC SUBSTANCES PRESENT IN GAS-PLANT WASTEWATER POND SLUDGE. **D.A. Birkholz, Enviro-Test Laboratories, Edmonton, AB, (403) 434-9509; G. Elliott, Environment Canada, Conservation & Protection, Edmonton, AB; R. Scroggins, Environment Canada, Conservation & Protection, Ottawa, ON.**

Sludge samples (40 L) were collected from the wastewater pond of a diethanolamine gas sweetening plant in Alberta. The sludge was dewatered by centrifuging at 4000 RPM and the resulting centrifugate and sludge samples were submitted to a toxicity identification evaluation (T.I.E.). A leachate was generated for the sludge (20 mL/g) using distilled water and both the leachate and centrifugate were subjected to toxicity testing using *Daphnia magna* and *Photobacterium phosphoreum* (Microtox). The centrifugate was observed to be more toxic than the leachate and hence efforts were focused on isolating and identifying the toxic components present in the centrifuge. Using solid phase extracting techniques coupled with fractionation of the resulting extract and subsequent toxicity testing, the most toxic components were isolated and identified. Variation from the published USA EPA T.I.E. procedures will be discussed.

APPLICATION OF THE THOMANN AND CONNOLLY AGE-DEPENDENT FOOD CHAIN MODEL TO PREDICT ORGANIC CHEMICAL CONCENTRATIONS IN AQUATIC ORGANISMS.

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From a water resource management perspective it is desirable to be able to estimate the potential impact of effluent constituents, on environmental concentrations and biological systems prior to their release rather than months, years or even decades later; in other words, be proactive rather than reactive. Ecosystem models, when used in conjunction with available environmental effect monitoring data enable informed decisions regarding actions that should be taken to manage ecological risks from areas of localized chemical loadings and accumulation. Aquatic organisms are exposed to chemicals in the environment through a number of routes, including exposure from the dissolved water column concentration and exposure from consumption of contaminated prey or sediment. Numerous biological, physical and chemical characteristics (*i.e.*, growth rate, molecular structure, K_{ow}) of the organism, chemical and the environment act together to determine chemical accumulation in the aquatic organism.

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs), accumulate in sediments and biological tissues, are environmentally persistent and extremely toxic to aquatic species, wildlife and humans. Consequently, the introduction of these compounds into the environment is a major concern and considerable efforts have been made to monitor PCDDs/PCDFs in the aquatic environment. The major exposure pathway of PCDDs/PCDFs is through consumption of contaminated food and sediments. The age-dependent food chain model (Thomann and Connolly 1989, updated 1991) was used to forecast PCDD/PCDF concentrations in aquatic species in response to PCDD/PCDF concentrations in the water column, suspended sediments, pore water, benthic sediments.

The age-dependent food chain model calculates chemical uptake from the water column via chemical transport across the gills relative to oxygen as a function of respiration rate and chemical diffusivity, and uptake through consumption of contaminated prey and sediments based on the chemical concentration in the food item, the food consumption rate and the chemical assimilation efficiency; chemical loss due to excretion and dilution from growth is also calculated. Total chemical uptake and accumulation can be modelled for both steady-state species (organisms for which the chemical body burden remains relatively constant) and age-dependent species (organisms for which the chemical body burden changes with the age, growth rate and dietary habits of each age-class). For a detailed discussion of the model theory and mathematical equations see Thomann and Connolly (1984a,b).

Briefly, the model allows the user to input biological dependent data describing the dietary interactions, initial weight, growth rate, fraction lipid, respiration, food assimilation efficiency for each species (steady-state or age-dependent) selected to be modelled. Chemical dependent data for the parameters K_{ow}, diffusivity ratio, and chemical assimilation efficiency are also input. One chemical assimilation efficiency can be input for all species or a value can be entered for each species individually; however for a given species the chemical assimilation efficiency is assumed to be constant regardless of the age-class. A measured fish/water bioconcentration factor (BCF) may be input directly for each species or can be calculated from the percent whole body lipid and the log K_{ow}. Finally, the model receives chemical concentration data for the water column dissolved (µg/L) and the sediment adsorbed (µg/g carbon).

The first step of any modelling exercise is to conduct a sensitivity analyses to determine the critical environmental parameters governing the model simulations, also referred to as scoping. The second step is to construct a site-specific aquatic food chain based on biological monitoring data for the aquatic system of concern, placing emphasis on key indigenous aquatic species at the various trophic levels. A general aquatic food chain consisting of phytoplankton, benthic invertebrate, pelagic invertebrate, bottom feeding fish, small fish and large fish was used to conduct a sensitivity analysis of the aquatic food chain model to the range of data describing the dietary assimilation efficiency of 2,3,7,8-T_4CDD and the log K_{ow} identified in the scientific literature. Data describing the dietary habits, biological parameters of selected aquatic species and chemical dependent parameters were taken from the published scientific literature. Dietary interactions of aquatic species will vary depending on the ecosystem, the general abundancy and availability of a potential food source, competition, seasonal changes in water temperature and other factors. In addition to feeding interactions, the use of growth rates and lipid content of populations inhabiting the study site increase the reliability of food chain model results on a site-specific basis. Organisms at the base of the food chain were considered to reach equilibrium relatively rapidly in the natural environment and adult stages of these species often do not differ significantly in concentration from earlier stages; therefore, these organisms were modelled as steady-state species.

Data required for the food chain model are listed in Table 1.

Table 1. Input Parameters Required for the Age-Dependent Food Chain Model

Steady-State Species	Age-Dependent Species
log K_{ow}	log K_{ow}
phytoplankton BCF	phytoplankton BCF
permeability ratio of chemical	permeability ratio of chemical
chemical assimilation efficiency	chemical assimilation efficiency
food assimilation efficiency	food assimilation efficiency
respiration rate	respiration coefficients
respiration temperature coefficients	fraction dry weight
fraction dry weight	growth rate for each age class
growth rate	initial weight for each age class
fraction of weight lipid	fraction lipid for each age class
chemical BCF for organism	chemical BCF for each age class
dissolved water concentration	predator-prey relations for each age
sediment concentration per g carbon	dissolved water concentration
predator-prey feeding structure	sediment concentration per g carbon

The scoping exercises conducted using the generic age-dependent food chain model to simulate 2,3,7,8-T_4CDD accumulation demonstrated that the model was highly sensitive to input describing chemical uptake at the base of the food chain (i.e. phytoplankton and suspended particulates) and the chemical assimilation efficiency of higher predators. Data available on the chemical assimilation efficiency of 2,3,7,8-T_4CDD in aquatic species was limited. Of that available the majority was for standard laboratory test fish species, rainbow trout, fathead minnow. A 2,3,7,8-T_4CDD assimilation efficiency of 0.34 was used for fish. Very little data was found in the literature for the assimilation efficiencies by invertebrates. Due to the lack of measured 2,3,7,8-T_4CDD assimilation efficiencies for aquatic invertebrates, a range of values (0.005 to 0.10) were tested for both pelagic and benthic invertebrates based on bioaccumulation data for various invertebrate species. In view of the model's sensitivity to data describing the chemical bioconcentration by phytoplankton and suspended particulates, as well as the chemical assimilation by benthic and pelagic invertebrates, further research is required to reduce the uncertainty surrounding the chemical uptake at the base of the food chain.

Upon validation of the food chain model simulations of PCDDs/PCDFs accumulation, the model can be used in conjunction with environmental fate models to estimate future concentrations of PCDDs/PCDFs in aquatic biota, to estimate the recovery period for a given habitat and fishery and to calculate potential PCDDs/PCDFs exposure levels of fish consumers including piscivorous wildlife and humans. Model validation requires comprehensive field monitoring data of concurrent PCDDs/PCDFS concentrations in the water column (dissolved), suspended sediment, sediment, pore water and biota, including invertebrates and fish. Methods of validation include a direct comparison between modelled and measured PCDD/PCDF concentrations in biota, calculation of sediment-to-biota accumulation factors (SBAF), and calculation of the bioavailability index (BI= the ratio of the lipid normalized chemical concentration in the organism to the organic carbon normalized chemical concentrations in the sediment).

Despite the considerable PCDD/PCDF environmental concentration data generated by both the federal government and industry, the majority of the available field monitoring data are insufficient for validation of both fate and food chain models and of little use for predicting future environmental transfer and food chain uptake and accumulation. Key data required for model verification that is often not readily available include: paired PCDD/PCDF concentration data for sediment and biota (collected within the same time frame); sediment organic carbon data; PCDD/PCDF concentration data for suspended solids, including phytoplankton; and PCDD/PCDF data for benthic and pelagic invertebrates. It is however, anticipated that these data may be available in the future as a result of more in depth monitoring programs designed to provide the necessary information for simulating and forecasting PCDD/PCDF concentrations in environmental media to be used in environmental assessments.

Potential applications of ecosystem models for the environmental assessment of hydrophobic chemicals such as PCDDs/PCDFs are numerous. For example, since it may take years for these compounds to accumulate in sediments and biota to detectable levels, the environmental impact of proposed changes in effluent PCDD/PCDF concentrations and corresponding environmental loading rates by conventional monitoring programs would take years to ascertain. The use of ecosystem models can provide immediate feedback of the estimated impact of proposed changes. In addition, the high cost (*e.g.*, $ 1000.00 per sample) of PCDD/PCDF analyses often prohibits in depth environmental monitoring. Scientifically validated ecosystem models can also be used to focus future environmental effects monitoring programs on sites of localized accumulation and investigate the impact that changes in effluent concentrations and new regulatory limits for compounds such as, PCDDs/PCDFs, in industrial effluents may have on environmental concentrations and their potential health effects on aquatic, piscivorous wildlife and humans consuming fish. Furthermore, the ecosystem model approach offers a scientific alternative to "zero discharge" presently proposed by some government and environmental groups.

Recently, Environment Canada and the Ontario Ministry of the Environment have recognized that existing water quality criteria do not adequately protect aquatic species and wildlife from hydrophobic chemicals, such as PCDDs/PCDFs, which have a high affinity for organic carbon of sediments and lipid material in biological tissues. Both organizations are developing guidelines for the determination of sediment quality criteria for the protection of aquatic and wildlife species. Once food chain model simulations have been verified with field data, the ecosystem model approach provides an invaluable tool to evaluate proposed sediment quality criteria for bioaccumulative and persistent chemicals.

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REQUIREMENTS FOR BENTHIC INVERTEBRATE SURVEYS DESIGN CONSIDERATIONS WITH A B.C. PERSPECTIVE.

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EXTENDED SUMMARY

National pulp and paper effluent regulations include a requirement for mill operators to conduct environmental effects monitoring (EEM) studies (DFO, 1992). Benthic invertebrate community surveys are required to assess the effects of mill effluents on fish habitat (Anon., 1992). The benthic invertebrate community assessment has two objectives: (1) to delineate the extent of habitat degradation due to organic enrichment or other forms of contamination, and (2) to provide an evaluation of the aquatic food resources available for benthivorous fish selected as sentinel species.

"Traditional" environmental assessment studies utilizing benthic invertebrates to quantify pulpmill related impacts have a long history in British Columbia. Some programs date back to the mid-1960's (Derksen, 1982). A retrospective look at some of these programs is useful for sample design considerations.

The hydrograph of a river can provide important background information with respect to the physical nature of a system. Eurocan Pulp and Paper at Kitimat discharge secondary treated kraft effluent into the Kitimat River several kilometres upstream of the estuary. The first low-flow period benthic invertebrate study was conducted in March 1989 (Wilkes et al., 1990). It is important to consider the river flow characteristics for the year the baseline data or impact assessment evaluation was conducted. In some cases it may require that future studies be scheduled such that comparable condition exists (Figure 1).

Several programs have evolved over a number of years and in some cases, procedural modifications have been made. The effect of some of these changes can be used to demonstrate the importance of having a quality assurance program that describes standard operating procedures (SOP) in a technical manual (EVS, 1992). Otherwise, some of these changes if not documented and demonstrated not to effect the integrity of the program, could result in the loss of valuable information for trend analysis or even result in misleading interpretations.

Weyerhaeuser Canada at Kamloops discharges secondary treated bleached kraft mill effluent into the Thompson River several kilometres upstream of Kamloops Lake. The company has conducted benthic invertebrate studies annually since 1978. The area of study and the habitat of concern is located downstream of Kamloops Lake. In 1983, the Walachin site was relocated several kilometres downstream due to access problems. The significance of this move was not evaluated at that time. In 1992, the old and new sites were both sampled. At the ordinal level, it appears that there are major differences in the abundance of ephemeropterans between the two sites (Figure 2). The change in sample site negates the usefulness of three years (1978 to 1980) of data in trying to understand what environmental variables may be controlling the benthic invertebrate community (Figure 3). The long-term nature of the Weyerhaeuser program is useful in the context of evaluating temporal variabilty in benthic invertebrate communities. As well, the Weyerhaeuser program provides an opportunity to assess the spatial variability in organism abundance over a number of years. The coefficient of variability (standard deviation/mean abundance x 100) over six years has varied between 30% to 94% with a median value of 44% (Figure 4). The Thompson River at Walachin is characterized by a stable substrate and stable flow during the winter period. Even under these "ideal" conditions there is a high level of variability associated with ephemeropteran abundance.

Antcliffe, 1991 assessed the Weyerhaeuser benthic invertebrate program from the context of sample size and statistical power. She estimated the number of samples required to measure a decrease in abundance at prescribed effect sizes (Figure 5). In order to measure a small effect size with an equally low probability of making either a Type I or Type II error, a large sample size is required and the size varies with the taxonomic group of interest.

Cariboo Pulp and Paper at Quesnel discharge secondary treated bleached kraft mill effluent into the Fraser River. The company has been conducting benthic invertebrate surveys for a number of years. Several procedural changes have been made over that time. Specifically, the two changes of interest include how the samples for detailed identification were treated and a change in mesh size (Figure 6). The richness index or "wealth" of taxa in a community was selected to determine if these procedural changes had any measurable effect. The procedural changes are reflected quite clearly by the richness index and has resulted in a loss of information that could be useful for long-term trend assessments (Figure 7).

There is one main lesson to be learned by a review of several of British Columbia's pulpmill benthic invertebrate studies. That lesson is that once the program objective has been defined and the methods and procedures agreed upon, changes to the program should not be made unless the effect of the change has been demonstrated and agreed upon by the concerned parties.

ACKNOWLEDGEMENTS

The data reviewed in this paper was collected by Weyerhaeuser Canada and Cariboo Pulp and Paper as a requirement of their Provincial Waste Management Permits.

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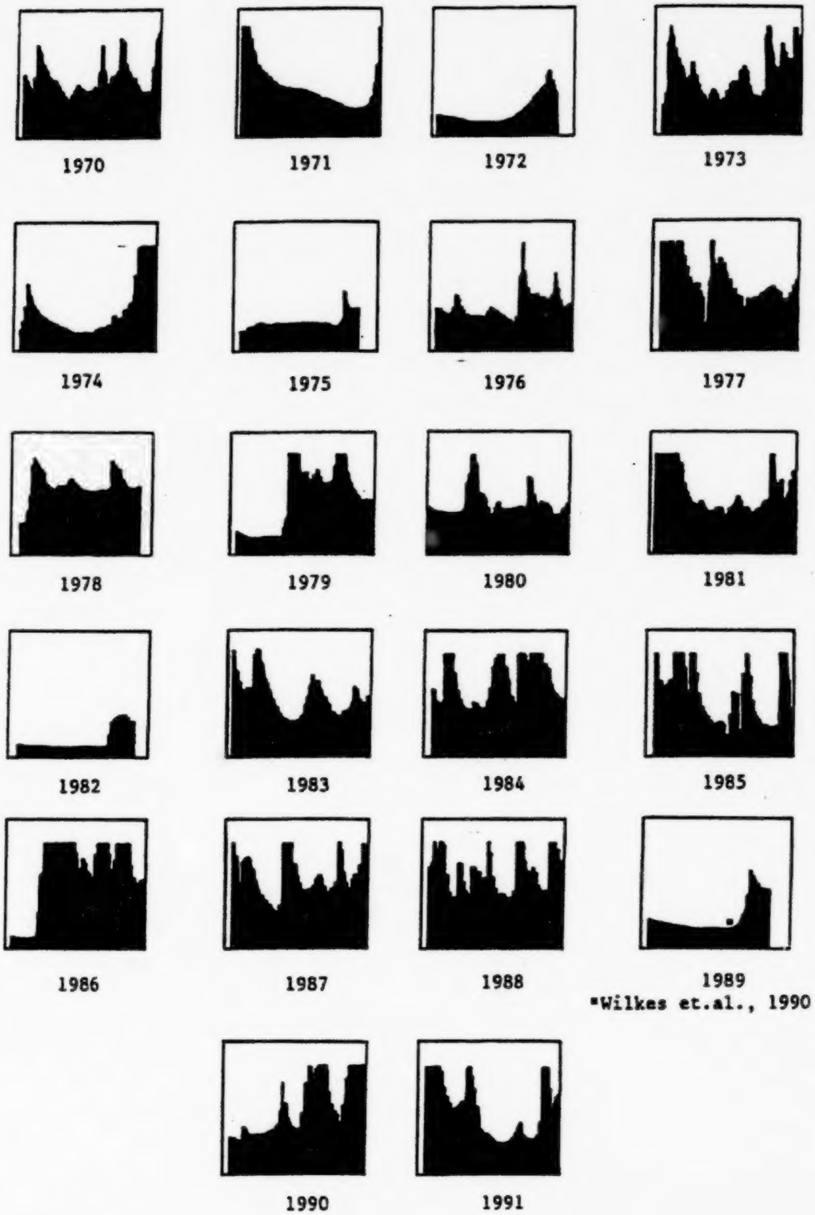


Figure 1. Kitimat River February-April Hydrograph, 1970-1991.

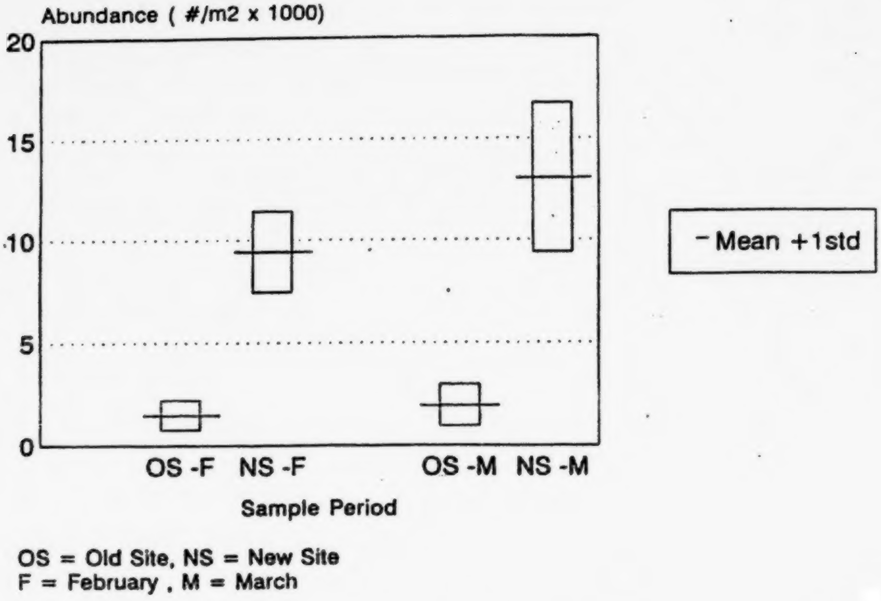


Figure 2. Weyerhaeuser Benthic Invertebrate Survey
Ephemeroptera Abundance - Walachin 1992

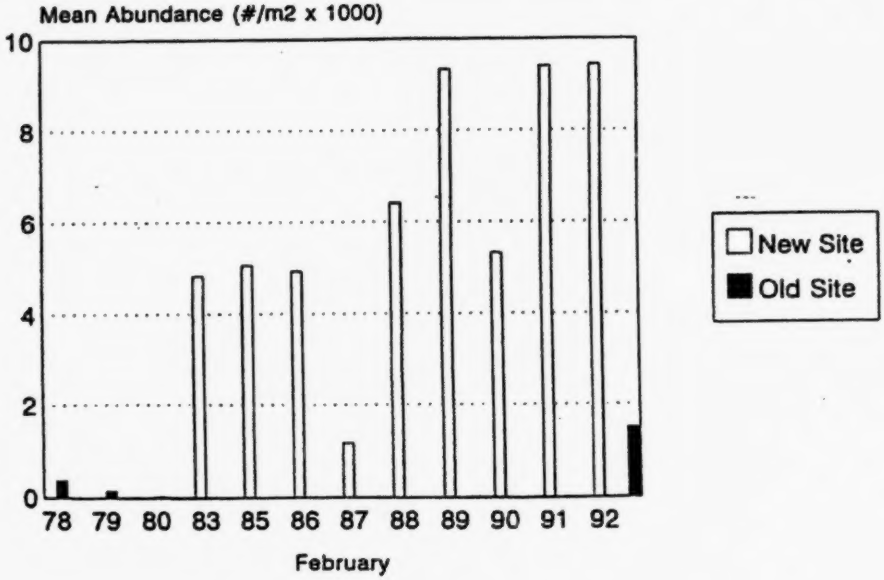
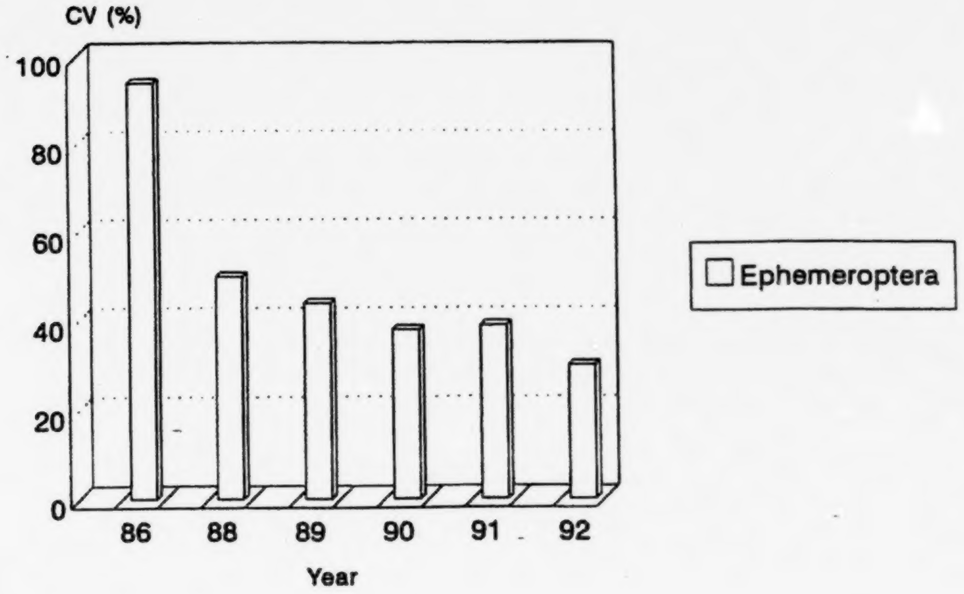


Figure 3. Weyerhaeuser Benthic Invertebrate Survey
Ephemeroptera Abundance - Walachin 1978 to 1992



n=12 for each year

Figure 4. Weyerhaeuser Benthic Invertebrate Survey Walachin Site - February + March

	EFFECT	SIZE	(decrease in abundance)
	40 %	50%	60%
Ephemeroptera	n=26	n=15	n=8
Trichoptera	n=178	n=99	n=56
Diptera	n=152	n=85	n=47

With High Power (0.95) and Alpha = 0.05, ln(x+1) transformation
Antcliffe, 1991

Figure 5. Sample Size Per Year Required for One-Way Anova to Detect a Change in Abundance of Benthic Invertebrates Weyerhaeuser - Walachin Site

	Sample Treatment For Richness Index*	Mesh Size
PERIOD 1 1975-1977	- Variable number of replicates used for detailed identification, >100 organisms required.	595um
PERIOD 2 1979-1981	- 4 to 5 replicates given detailed identification and N = mean total abundance	595um
PERIOD 3 1982-1987	- 4 to 5 replicates given detailed identification and N = mean total abundance	180um

R = S-1/log e N : S = # taxa per sample; N = total # individuals per sample.

Figure 6. Cariboo Station 1 Richness Index

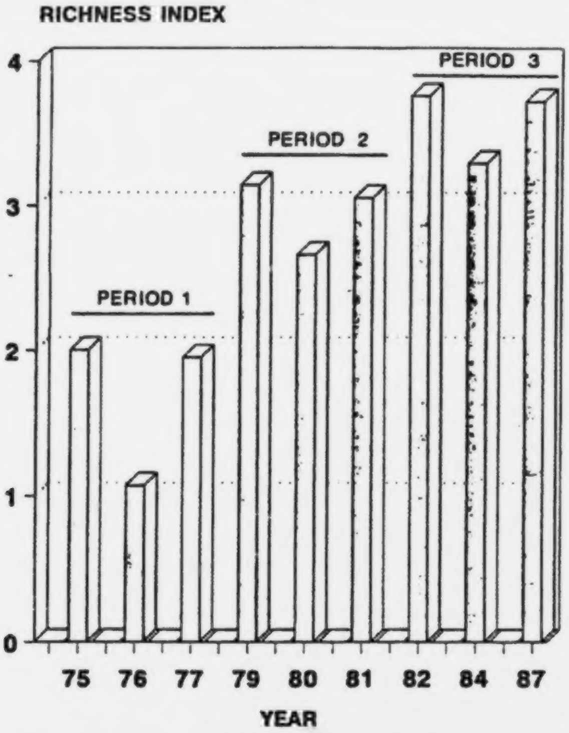


Figure 7. Cariboo Benthic Invertebrate Survey Station 1 - Fall Tray Data

DO SEWAGE DISCHARGES FROM VICTORIA (BC) POSE A MAJOR ENVIRONMENTAL PROBLEM? - TOXICITY AND RELATED STUDIES.

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ABSTRACT

Municipal wastes from the City of Victoria, BC and environs are screened, then discharged to the sea as effluent from two long (1154 and 1800 m) outfall pipes (Macaulay and Clover Points). The question of whether the effluent should be further treated has become a national and international issue. However, prior to this study, adequate data were not available to fully determine the extent and severity of any outfall related effects. Sediment chemicals, toxicity and benthic communities were determined for Macaulay Point. Bioaccumulation of chemicals was determined for Clover Point using a mussel which lives in the hard bottom around that outfall. Sewage toxicity studies were conducted at both outfalls. Effluent toxicity was generally similar between the two outfalls; low dissolved oxygen and ammonia were the primary "culprits" for the observed toxicity; water column toxicity after discharge was generally restricted to a few meters from the outfall pipe. Outfall-related chemicals were not bioaccumulated to levels of concern. Sediment toxicity was restricted to effects on growth and development at the Macaulay Point outfall and at stations 100-400 m away; survival was near control levels in sediments even at the outfall terminus. Benthic infaunal communities showed a classic organic enrichment pattern at Macaulay Point of low species richness and high abundance near the outfall, increasing species richness and lower abundance with distance from the outfall; the outfall terminus was classifiable as "moderately polluted"; effects were limited to the terminus and to 100 m stations. Sediment biological effects and chemical contamination were greatest at and near the outfall, and decreased rapidly with distance in any direction (generally to background levels within 100-400 m).

EXTENDED SUMMARY

The majority of municipal wastes (liquid or municipal waste water) from the City of Victoria, BC and environs are discharged to the sea as effluent from two long outfall pipes: Macaulay Point (1,800 meters offshore at a depth of 61 m) and Clover Point (1,154 meters offshore at a depth of 67 m). The effluent is passed through screens to remove larger particles and minimize clumping, but not otherwise treated. Dilution after discharge is maximized by the use of diffusers, consisting of small holes in the sidewall of the end piece of outfall pipe. Once released, the effluent rises because, since it is primarily fresh water, it is more buoyant than the surrounding salt water. Mixing of salt water and effluent occur immediately upon release and continue as the effluent moves through the water column. The effluent stops rising when its buoyancy matches that of the surrounding water. This point of neutral buoyancy is referred to as the "trapping depth". During summer, when lower salinity waters are found on the sea surface due to natural oceanographic processes, the plume does not reach the surface. In winter, when there is little difference between the top and bottom of the water column at the point of discharge, the effluent plume may, rarely, reach the surface. As the plume rises it is usually moved in one direction or another due to currents in the area. This movement, which increases the dilution of the effluent, is called dispersion.

Several processes occur as soon as the effluent is released and continue as the effluent rises and is dispersed:

- particulates and materials associated with particulates drop out and are deposited on the sea bottom

- fats float upward, generally to the surface (sea gulls on the surface in the area of the outfall terminus feed on drops of fat which have congealed in the cold sea water)

- marine organisms in the area are exposed to the effluent, with the highest exposure occurring with the highest effluent concentration, that is, closest to the outfall.

The fact that the effluent is not treated beyond screening whereas most other municipal effluent discharges in North America receive further treatment has been a source of concern on both sides of the USA-Canada border to some members of the public, regulators and politicians. Political and other pressures have been applied to the Capital Regional District (CRD) to further treat the effluent. Such treatment could involve:

- **Source Control** - Involves reducing chemical loadings in the final effluent by controlling the sources of such inputs. Source control efforts commonly target key industrial, commercial and institutional groups.

- **Primary Treatment** - Involves physical operations such as screening (already being done) and sedimentation to remove readily settleable or floating solids, thereby reducing oxygen demand and reducing concentrations of: pathogens, oil and grease, and suspended solids. However, primary treatment will not appreciably reduce toxicity (e.g., due to ammonia) nor will it appreciably reduce the concentrations of chemicals not associated with readily settleable or floating solids. The material removed becomes sludge, which must then be disposed of.

- **Secondary Treatment** - Involves additional chemical or biological treatment following primary treatment, and serves to remove most of the suspended and soluble organic matter. Secondary treatment will further reduce oxygen demand and pathogens and, more importantly, it will also appreciably reduce concentrations of some chemicals. However, some forms of secondary treatment will not appreciably reduce toxicity (e.g., due to ammonia) nor appreciably reduce the concentrations of dissolved chemicals or those which are not associated with readily settleable or floating solids. The material removed becomes sludge, which must then be disposed of.

- **Tertiary Treatment** - Constitutes any type of treatment beyond secondary and is site- and situation-specific. Tertiary treatment may, for example, include additional removal of nutrients or of oxygen demanding materials. Tertiary treatment in the form of, for instance, effluent filtration, would further reduce chemical concentrations in the effluent. However, as is the case for any level of treatment, material removed from the final effluent must then be disposed of.

It is generally accepted that any additional levels of treatment beyond preliminary treatment such as screening should be designed to solve real environmental problems resulting from the two outfalls. However, although there is good evidence for lack of effluent-related impacts in the water column, to date it has not been clear to what extent the outfalls are actually impacting the receiving sedimentary environment. This uncertainty has resulted from the fact that monitoring to date has primarily been conducted in the water column, with little examination of effects to biological communities on the seafloor:

- Regular effluent monitoring has, in the past, been restricted to physical measurement of flow and to four conventional water quality parameters: biological oxygen demand, BOD; total suspended solids, TSS; ammonia; and, bacterial contamination in the form of fecal coliforms. Priority pollutant analyses of the effluent are being done annually but will be done quarterly from August 1992.

- Receiving environment monitoring has been generally restricted to fecal coliforms in the water column, limited sediment chemical analyses 1985-1989, and more detailed analyses since 1988.

- Special studies and monitoring conducted by the CRD have not fully characterized contamination or effects on resident communities of organisms in the sediments.

- The toxicity of the effluent and any toxicity in the receiving sedimentary environment have not been characterized.

The purpose of the present study was to address, to the maximum extent possible, uncertainty regarding the effects of the two outfalls on the marine sediment receiving environment. Although uncertainty regarding possible but undetected adverse effects can never be totally eliminated from studies of effluent or other discharges (or, in fact from any aspect of human existence as there are an infinite number of improbable but possible "What if?" scenarios), it can be minimized. Uncertainty was minimized by concentrating on "worst case" measurements at the two outfalls as follows:

- Laboratory tests were conducted to determine the toxicity of the actual effluent. A 24-h composite sample was tested as well as a grab sample to ensure that tests were based on reasonably representative effluent samples (single grab samples may miss brief but significant "peaks" in chemical loadings). These tests involved determining the survival of two freshwater organisms (rainbow trout and a waterflea [*Ceriodaphnia dubia*]) and the ability of Pacific oyster and blue mussel bivalve larvae to develop from a fertilized egg to a fully shelled larval stage. Compared to real-life exposures to resident organisms, these tests provided "worst case" responses: the organisms had no prior chance to adjust to, nor to escape from, the effluent.

- After release from the diffuser ports, the effluent is rapidly dispersed. However, persistent chemicals will accumulate in the sediments. Thus, the sediments provide a long-term record of contamination and of any adverse biological effects due to persistent chemicals from the outfalls. Accordingly, receiving environment testing was done on the sediments and/or organisms associated with the sediments. At the Macaulay Point outfall, where the sediment consists of muds, both the sediments and the organisms living in the sediments were studied. At the Clover Point outfall, where the bottom consists of rock and cobble, a representative organism living at and around the outfall was studied. In both cases, studies were conducted immediately off the mouth of each outfall ("worst case"), along gradients to east and west of the outfall (and to the southwest at Macaulay Point), and at reference site(s) well removed from the immediate influence of the outfalls. Stations along the gradients were located at distances of 100, 400, 800 and up to 1600 m from the outfall discharge; the reference sites were located several to tens of kilometres away. The following studies were conducted at each outfall:

Macaulay Point

1. Sediment toxicity tests - sediments were collected and returned to the laboratory where the responses of three different organisms to the sediments were determined: the ability of a common juvenile marine worm (*Neanthes arenaceodentata*) to live and grow over a 20 day exposure period; the ability of an adult amphipod (*Rhepoxynius abronius*) to survive over a 10 day exposure period; the ability of mussel larvae (*Mytilus edulis*) to develop from a fertilized egg to a fully shelled larval stage. The larval test was also used for the effluent toxicity tests as noted previously. As was the case with effluent toxicity testing, compared to real-life exposures to resident organisms, these tests provided "worst case" responses: the organisms had no prior chance to adjust to the effluent.

2. Sediment chemistry analyses - the same sediments used for toxicity testing were analyzed to determine what chemical contaminants were present. Chemical analyses were directed towards chemicals found in the effluent which could be accumulating in the sediments and causing adverse biological effects.

3. Benthic organism community analyses - at the same time and in the same location as sediments were collected for toxicity testing and chemistry analyses, additional sediments were collected, sieved, preserved and analyzed to determine what was actually living in the sediments at these locations.

Clover Point

1. Due to the nature of the sea bottom at Clover Point (rocky and hard), it was not possible to collect sediment samples. Therefore, a common species of filter-feeding mussel (*Modiolus modiolus*), was collected, the shell removed, and the animal was then analyzed for chemicals in tissues, much as was done for sediment chemistry analyses at Macaulay Point. Several individual animals were combined at each site and analyzed to ensure that the results were representative of the population. At

Clover Point, as at Macaulay Point, most of the material from the outfall does not settle but, rather, remains in the water column where it may be available to organisms such as this mussel. Thus, analyses of tissues from this organism provided "worst case" data for bioaccumulation of contaminants from the outfall effluent.

The results of the above studies were subjected to detailed statistical and other data analyses. The major findings were as follows:

Macaulay Point and Clover Point Effluent Toxicity

The following range of effluent toxicity was observed at the two outfalls for the two test periods (expressed as % effluent diluted with clean laboratory water):

- Rainbow trout 96-h LC50: 42 to 64%
- *Ceriodaphnia* 48-h LC50: 1 8 to >100%
- Bivalve (oyster and mussel) larvae 48-h EC50: 1.1 to 3.9%; NOEC: <0.2 to 1.6%.

With the exception of the *Ceriodaphnia* test, effluent toxicity was remarkably similar between the two outfalls and the two test periods. The reason(s) for the greater range of response with the *Ceriodaphnia* cannot be determined from this study.

Low (toxic) dissolved oxygen levels and high (toxic) ammonia concentrations in the effluent were most likely responsible for at least some of the observed toxicity. These causal factors are not persistent and will be rapidly rendered harmless with dilution upon discharge. Whether some of the observed toxicity was also due to more persistent agents could not be directly determined from this study, but was indirectly addressed through sediment toxicity testing (persistent toxicants tend to accumulate in sediments). However, modelling indicated that any water column toxic effects would be restricted to, at worst, a few tens of square meters around the outfall pipe, well within the 100 m initial dilution zone (IDZ) allowed under the CRD's Provincial permits.

Macaulay Point Sediment Toxicity

The results of the sediment toxicity testing indicated that all sediments, even those immediately off the outfall terminus, are capable of supporting life. However, the sediments closer to the outfall (that is, at the terminus and at 100 m distances) showed significant adverse effects. The range of responses noted at all stations was as follows:

- 65-98% of amphipods survived for the 10-d exposure period
- 76-100% of the worms survived for the 20-d exposure period and growth rates were 33-115% of those in the controls
- 48-86% of the mussel larvae survived for the 48-h exposure period; 37-83% of the larvae showed normal development from fertilized eggs to fully shelled stage.

In contrast to the other two tests, the mussel larvae test showed significantly higher toxicity than the control for all sediments tested, including those from the Parry Bay reference stations. It is extremely unlikely that toxicity in these far-field sediments is associated with the outfall because of the pattern of sediment contamination and results of benthic community structure determinations, as discussed below.

Macaulay Point Sediment Chemistry

Sediment chemical analyses indicated that the highest chemical concentrations associated with the outfall and capable of causing adverse biological effects occurred at the outfall terminus and at the 100 m stations. Mercury and 1,4-dichlorobenzene (1,4 - DCB), were present in these sediments at concentrations which could cause adverse biological effects.

The polyaromatic hydrocarbons (PAH) were present at highest concentrations to the east of and at some distance from the outfall. However, elevated concentrations of these compounds were not due to discharge from the outfall but were, rather, attributable to coal and coke in the sediments, apparently the result of the shipwreck of a collier in the 1890s. With the exception of the coal and coke-related PAH, no chemicals exceeded the CRD sediment guidelines (limits or standards) beyond the 100 m stations.

Macaulay Point Benthic Community Structure

A total of 394 taxa were identified. Benthic community structure showed a classic pattern of organic enrichment effects close to the outfall, including low species richness and high total abundance of organisms. These effects decreased with distance from the outfall and were most pronounced at the terminus and 100 m stations. The polychaete worm, *Capitella capitata*, was particularly abundant near the outfall, which is in accord with this opportunistic species' predilection for organically enriched and disturbed conditions. The outfall terminus and 100 m stations are classifiable as "moderately polluted" based on the classical organic enrichment model.

Clover Point Tissue Chemistry

Tissue chemical concentrations in the mussel, *Modiolus*, were generally similar, that is, within a factor of two, at the Ten Mile Point reference station and at the outfall stations. Surprisingly, there was no consistent pattern of higher chemical concentrations near the outfall. For instance, while the PAH and lead concentrations were highest at the outfall terminus and then decreased with distance from the outfall, the reverse was true for mercury. 1,4-dichlorobenzene was not detected in the mussel tissues.

Based on the results of this study, five primary questions were answered related primarily to the Macaulay Point outfall:

Question 1: *Are present sewage discharges impacting the local marine environment?*

Answer: Yes.

Question 2: *If so, what is the impact(s)?*

Answer: The impacts appear to be minimal, specifically: (1) increased abundance and reduced diversity of benthic organisms near the Macaulay Point outfall; and, (2) increased toxicity near the Macaulay Point outfall resulting primarily in reduced growth and development of laboratory organisms exposed to sediment from this area.

Question 3: *If so, what is the extent of the impact(s)?*

Answer: The impacts which can be directly related to the Macaulay Point outfall are generally contained within a 100-400 m area around the outfalls (effects occur at 100 m but generally not at 400 m; there were no intermediate stations).

Question 4: *What is the environmental significance of any observed impacts?*

Answer: There do not appear to be any major adverse effects on the receiving environment at Macaulay Point outside of the initial zone of dilution (an area of 100 m around the outfall allowed under the CRD's present Provincial discharge permit). At Clover Point impacts will be more diffuse due to the higher currents (hence greater dispersion) and non-depositional sedimentary environment.

Question 5: *Can any impacts be attributed to specific contaminant(s) and, if so, which one(s)?*

Answer: No. Mercury and 1,4-dichlorobenzene are highly elevated in the area where effects are observed at Macaulay Point and are primary candidates for source control. However, 1,4-dichlorobenzene is not bioaccumulated by mussels at Clover Point and the pattern of mercury bioaccumulation is not centred on the Clover Point outfall.

FINAL COMMENTS

The results of this study form part of the information presented to area residents prior to a public referendum (November 1992) on the issue of sewage treatment. The present study was only scientific. Because treatment decisions incorporate many non-scientific elements, specific recommendations as to level(s) of additional treatment are not made.

INTER-LABORATORY COMPARISON OF AN APPROACH FOR SCREENING OF PULP AND PAPER MILL EFFLUENTS WITH RESPECT TO THEIR ABILITY TO CAUSE ELEVATED MIXED FUNCTION OXYGENASE (MFO) ACTIVITY IN FISH.

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ABSTRACT

Direct exposure to effluents under laboratory conditions was identified as a practical approach for screening mill effluents for their ability to cause elevated ethoxyresorufin-O-deethylase (EROD) activity in fish. The approach was used at PAPRICAN and Environment Canada. The objective was to determine if comparable results could be achieved despite differences in exposure conditions such as loading density, fish size, test volume and EROD methodology. Rainbow trout *(Oncorhynchus mykiss)* were exposed for four days to a 10% concentration of a secondary-treated bleached kraft mill effluent under static conditions with daily renewal. Control groups were exposed to dilution water. Exposures were initiated simultaneously at both laboratories. At the conclusion of the exposure, frozen post-mitochondrial supernatant samples were exchanged for EROD analysis. Results show that although differences exist in terms of absolute EROD activity, both laboratories measured significant EROD induction (Figure 1). It may be concluded that the results obtained by both laboratories are comparable.

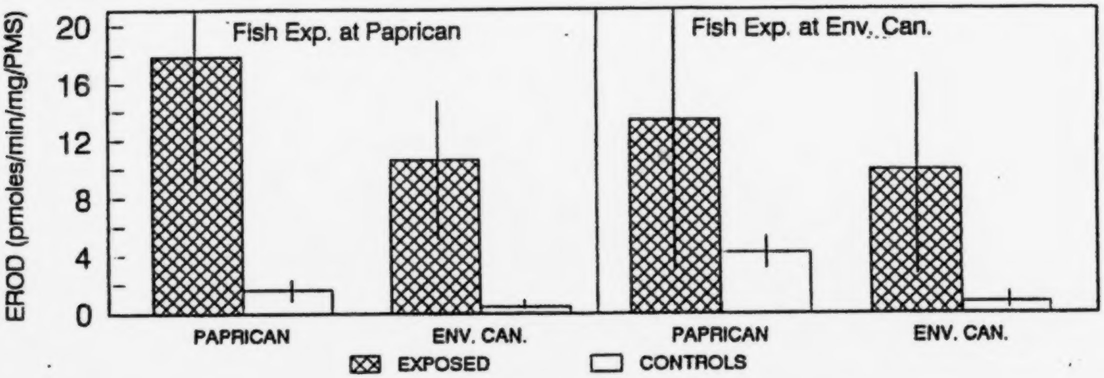

Figure 1. The laboratories tested identical BKME and analyzed both sets of livers from 10% exposures for 4 days. Paprican used 96-g trout in 150-L aquaria. Environment Canada used 13-g trout in 10-l aquaria.

ISOLATION BY NANOFILTRATION AND CHARACTERIZATION OF HIGH MOLECULAR SIZE (>400) ORGANIC MATERIAL FROM BLEACHED KRAFT PULP AND PAPER MILL EFFLUENT.

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ABSTRACT

Bleached kraft mill effluent (BKME) was fractionated by nanofiltration to a colourless low molecular size (<400 Dalton) fraction and a strongly coloured high molecular size (>400 Dalton) fraction. The filtration membrane (Filmtec NF40-40) did not reject chlorophenolics, but rejected 99% of the sulphate, 10% of the chloride, 45% of the monovalent cations and 80% of the divalent cation. The high molecular size (HMS) fraction contained 87 to 97% of the carbon adsorbable organic chlorine (AOX) and 72 to 99% of the dissolved organic carbon (DOC) with carbon to chlorine ratios from 12 to 18. Permeate flux decreased over time due to fouling unless the membrane was cleaned periodically with a base solution (P3 Ultrasil 10). Flow rates were maintained to approximately 12 litres/hour/meter^2 (4.5 litres/min.). Nanofiltration allowed the isolation of lower molecular size organics than possible with tangential flow ultrafiltration (1000 D) without the high salt rejection characteristics of reverse osmosis membranes. This unit is also adapted to carry in the field and can process up to 1400 litres of effluent daily.

INTRODUCTION

The use of membrane methods such as ultrafiltration (UF), reverse osmosis (RO) and nanofiltration (NF) for the treatment of kraft mill effluent is a well researched field. In this work, we investigated the use of nanofiltration as a tool to isolate concentrate and purify high molecular size organic matter from kraft mill effluents. Nanofiltration is a pressure driven membrane process with lower ion rejections characteristics than RO and lower molecular weight cutoffs than UF. Presented here is the description of an effluent fractionation method and the performance results of the NF40-40 (Filmtec, Minneapolis, MN) membrane when used to isolate organics from two bleached and one unbleached kraft mill effluent.

MATERIALS AND METHODS

A maple sap concentrator (Les Equipments Lapierre inc.) was modified by using a NF40-40 (FilmTec, Minneapolis, MN) membrane and a greaseless ceramic piston high pressure pump (X-8, Magikist Ltd.) (Figure 1). The NF40-40 membrane had a nominal molecular weight cutoff of 400 Dalton. Prior to nanofiltration, the effluent was centrifuged (Westfalia cream separator) at 4 to 6 L/min and filtered through a glass fibre filter (Gelman A/E, 142 mm diameter, 1 micron pore size) to remove the suspended solids and reduce membrane fouling. The nanofiltration unit was operated at constant membrane pressure of 250 psi (1.7 MPa) and the concentrate flow was maintained at 4.45 L/min. Usually 600 litres of effluent were concentrated 50 % in the transfer mode (Figure 2A). The high molecular size organic matter was further concentrated in the recirculation mode to approximately 10 litres (Figure 2B). This concentrate was drained into a stainless steel container and depending on permeate flow rates, the membrane was cleaned with either permeate, 0.1% NaOH with 0.025% SLS or 1.0% solution of P3 Ultrasil 10 (conditions: 0 psi and concentrate flow rate > 4.5 L/min.).

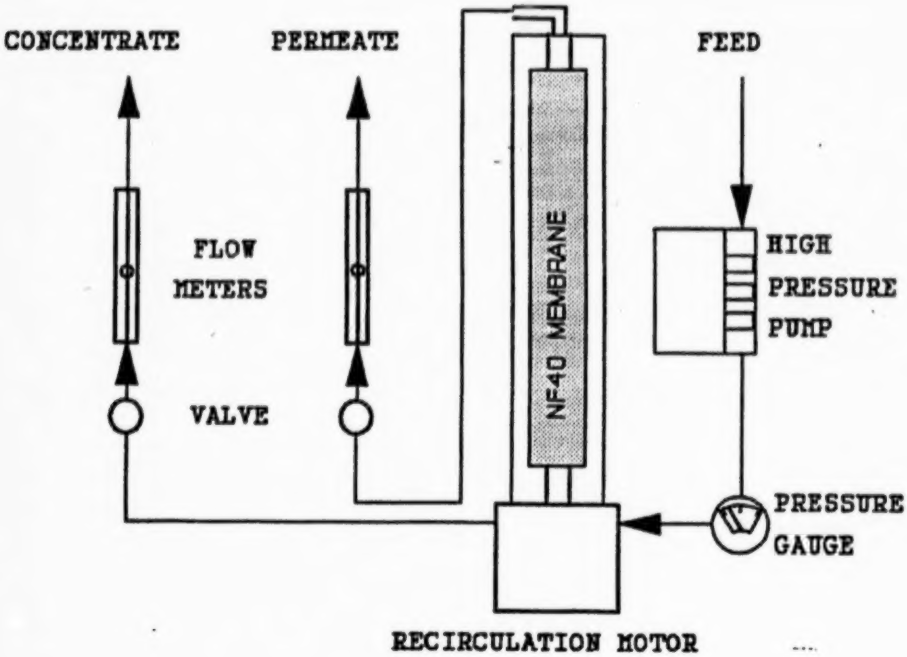
CONCENTRATE
PERMEATE
FEED
FLOW METERS
VALVE
NF40 MEMBRANE
HIGH PRESSURE PUMP
PRESSURE GAUGE
RECIRCULATION MOTOR

Figure 1. Nanofiltration unit

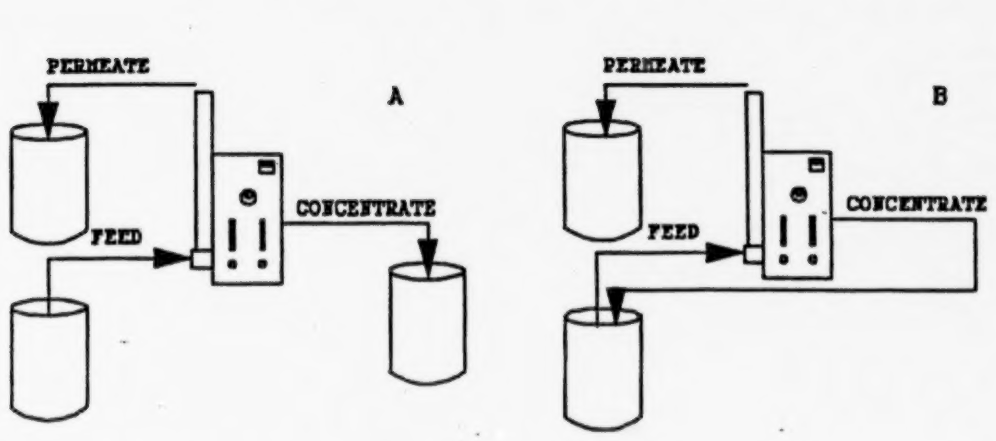

Figure 2. A. Transfer mode B. Recirculation mode

RESULTS AND DISCUSSION

Unbleached and bleached kraft effluent was successfully fractionated using a reverse osmosis unit equipped with a NF40-40 nanofiltration membrane. The reverse osmosis unit allowed the fractionation of up to 1400 litres of effluent daily. This feature makes the method very attractive for long term studies where a consistent source of high molecular size organics is needed. This unit is also adapted to carry in the field which is sometimes needed to eliminate the possible effects of shipping the effluent to the laboratory.

Membrane ion rejection was monitored during the first effluent fractionation trial. Rejection values were 99% for sulphate, 10% for chloride, 45% for monovalent cations (sodium and potassium) and 80% for divalent cations (magnesium and calcium). These values were in accordance with the membrane specification provided by Filmtec (Minneapolis, MN). However, during this trial permeate flow rates decreased drastically due to membrane fouling. This demonstrated that ion rejection could not be used to monitor the membrane performance since fouling did not appear to affect the ion rejection properties of the membrane. The preferred method to assess membrane performance was by monitoring the permeate flow rates during the fractionation. Several cleaning agents were tested for their ability to prevent membrane fouling. Cleaning with effluent permeate or with a solution of 0.1% NaOH with 0.025% SLS was insufficient to prevent fouling and maintain permeate flow rates. When the membrane was cleaned periodically with a 1% solution of P3 Ultrasil 10, flow rates could be maintained to approximately 12 litres/hour/meter^2 (4.5 litres/min.). Chlorophenolics such as 2,3,4,5-tetrachloroguaiacol, 3,4,5-trichloroguaiacol, 4,5,6-trichloroguaiacol and 2,4,6-trichlorophenol were not rejected by the nanofiltration membrane. The behaviour of the less chlorinated phenolics during fractionation is still unclear. It was also found that extensive diananofiltration could remove up 99% of the chlorophenolics from the >400 Dalton fraction. The high molecular size (HMS) fraction contained 87 to 97% of the carbon adsorbable organic chlorine (AOX) and 72 to 99% of the dissolved organic carbon (DOC) with carbon to chlorine ratios from 12 to 18 (Table 1).

Table 1. High molecular size (>400 D) dissolved organic carbon (DOC) and adsorbable organo-halogen (AOX) distribution in unbleached (UKME) and two bleached kraft mill effluents (BKME)

	% >400 Dalton			
	DOC	AOX	C:Cl	Source
BKME 1	83.0	88.9	18.1	Primary Treatment
UKME	77.4	N/A	N/A	Primary Treatment
	72.7	N/A	N/A	Primary Treatment
	87.4	N/A	N/A	Secondary Treatment
	82.8	N/A	N/A	Secondary Treatment
BKME 2	82.4	86.7	12.0	Primary Treatment
	82.8	89.6	16.4	Primary Treatment
	99.1	97.6	13.0	Secondary Treatment
	98.0	94.5	11.6	Secondary Treatment

CONCLUSION

Large volumes (1400 L/day) of kraft effluent can be fractionated by nanofiltration into a colourless permeate and highly coloured concentrate. The nanofiltration unit is well adapted to bring in the field. Membrane cleaning with 1% P3 Ultrasil 10 is recommended to maintain permeate flow rates. Ion rejection values are not a good indicator of membrane integrity. Low molecular size chlorophenolics such as 2,3,4,5-tetrachloroguaiacol, 3,4,5-trichloroguaiacol, 4,5,6-trichloroguaiacol and 2,4,6-trichlorophenol were not rejected by the nanofiltration membrane.

INDUCTION OF MIXED FUNCTION OXIDASES AND CYTOCHROME P-450 IN LIVER OF MATURE RAINBOW TROUT.

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ABSTRACT

Bioindicators of fish exposure to chronic or sublethal concentrations of xenobiotic chemicals can be valuable for hazard assessment. The aim of this investigation was to study the biochemical aspects of benzo(a)pyrene hydroxylase (BPH) and 7-ethoxycoumarin-O-deethylase (ECOD) for their use as sensitive bioindicators of xenobiotic effects in rainbow trout under initially controlled environmental conditions. Mature fish were administered (i.p.) a single dose of (i) 3-methylcholanthrene (3-MC) in corn oil (12.5 $mg{\cdot}kg^{-1}$), or (ii) corn oil (control for 3-MC), or (iii) phenobarbital (PB) in saline (100 $mg{\cdot}kg^{-1}$), or (iv) saline (control for PB), and sacrificed 96 hours post dosing. Marked induction of BPH and ECOD activities and a significant increase in cytochrome P-450 (P-450) were observed in hepatic microsomes of only 3-MC treated fish. Further studies showed that in 3-MC treated fish both enzymes were significantly induced within 24 hours after dosing. In these fish, the activity of BPH remained significantly elevated even two weeks after dosing. Microsomal preparations from control and 3-MC treated fish showed (i) no change in BPH activity upon storage at 0-4°C for 2-3 days or in a frozen state, and (ii) marked reduction in ECOD activity upon storage. Furthermore, addition of α-naphthoflavone and metyrapone, two differential inhibitors of P-450 isoenzymic forms, to BPH assay affected this enzyme's activity quite distinctly in microsomes of 3-MC treated and control fish. Future research will assess the value of these biochemical parameters for monitoring the effects of pulp mill effluent under field and controlled exposure conditions.

In an aquatic environment fish can be continuously exposed to a variety of chemicals discharged by industrial operations in chronically low or sublethal concentrations. The presence of such xenobiotic chemicals in receiving water bodies is an important regulatory concern especially for downstream of major industrial plants such as pulp mills. Bioindicators of fish exposure to xenobiotics causing specific biochemical changes can be very valuable for hazard identification and assessment of related toxicity.

This study was conducted primarily for (i) validating the induction characteristics of two mixed function oxidase (MFO) activities in the liver of mature rainbow trout and (ii) developing simple and fast procedures to identify the isoenzymic nature of induced changes.

Materials and Methods

Study Design

Mature (body weight >250 g) rainbow trout (*Oncorhynchus mykiss*) were injected intraperitoneally with a single dose of 3-methylcholanthrene (3-MC) or its vehicle (corn oil), or of phenobarbital (PB) or its vehicle (0.9% NaCl) at the following dosage levels:

3-MC - 12.5 $mg{\cdot}kg^{-1}$ body weight
PB - 100 $mg{\cdot}kg^{-1}$ body weight

Collection and Processing of Samples

The trout were sacrificed at specified time intervals post injection and microsomal preparations were made from liver tissues by standardized procedures (Schuler and Khan, 1991).

Enzyme Assays

7-Ethoxycoumarin-O-deethylase (ECOD), benzo(a)pyrene hydroxylase (BPH) and cytochrome P-450 (P-450) were analyzed according to Khan et al. (1989).

Studies with Differential Inhibitors

The activity of BPH was assayed in the presence of 1.0 $\mu mol \cdot L^{-1}$ α-naphthoflavone (α-NF) and metyrapone.

Results

Stability of MFO in Stored Microsomes

BPH activity and P-450 content in rainbow trout microsomes were stable in storage in the cold (0-4°C) for 2-3 days and for several weeks at -50°C. However, ECOD activity was very unstable upon storage at either temperature.

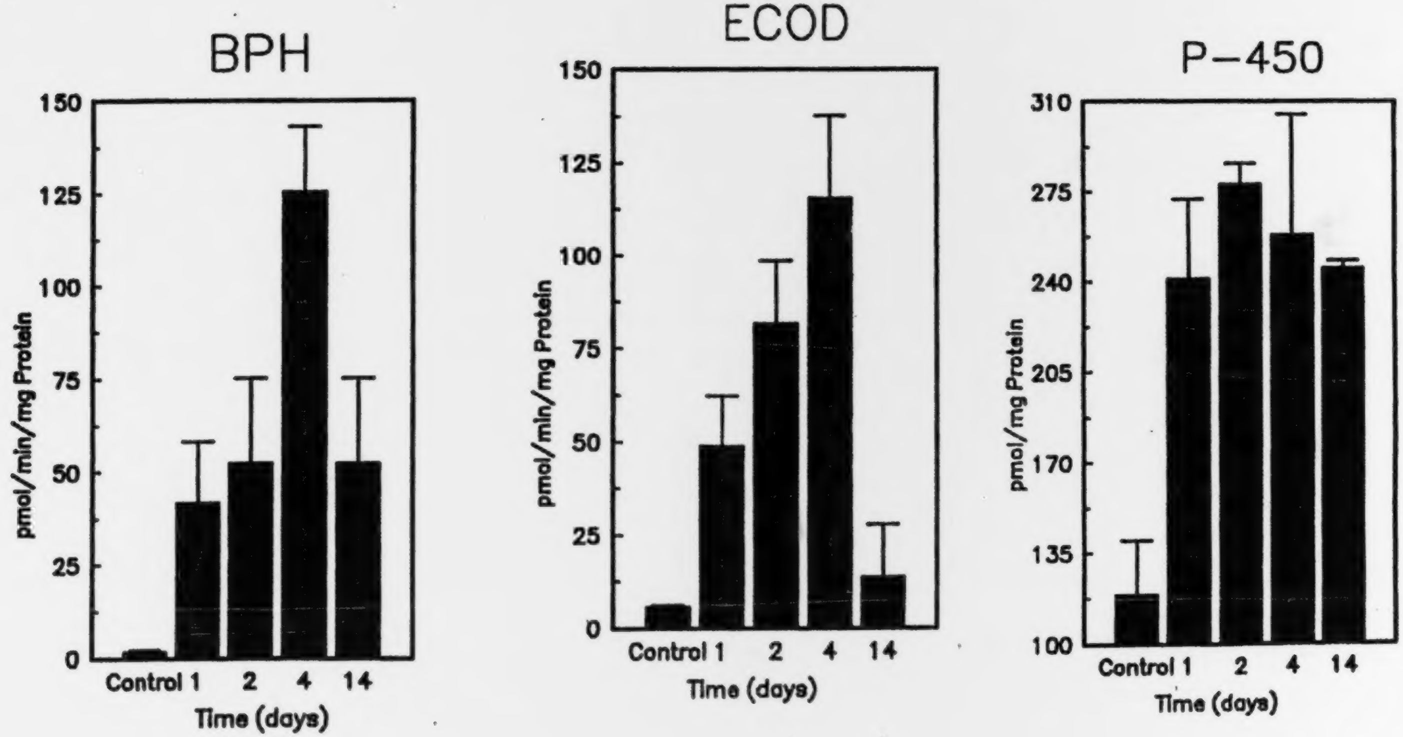
Effects of 3-MC and PB on Hepatic P-450 and MFO Activities of Rainbow Trout

Analysis of P-450, BPH and ECOD showed marked induction within the first day after treatment with 3-MC (Figure 1). Treatment with PB showed no significant change in these biochemical parameters. In the 3-MC treated fish these parameters were maximally induced on Day 4. BPH showed the greatest induced change (>120 fold) followed by ECOD (>20 fold) and P-450 (≈2 fold). The activity of BPH remained significantly elevated even after 14 days post dosing. Analysis of P-450 showed a distinct spectral shift from 450 nm (control) to 448 nm in fish treated with 3-MC (Figure 2).

Effects of Differential Inhibitors

Microsomal samples from control and 3-MC treated fish showed markedly different effects of α-NF and metyrapone on BPH activity. In 3-MC induced samples α-NF showed a very powerful inhibition of BPH (>90%) while metyrapone had no significant effect. Control (uninduced) samples exhibited inhibition by both α-NF and metyrapone; however the effect was more marked with α-NF.

Figure 1. Temporal Assessment of Induction by 3-MC.



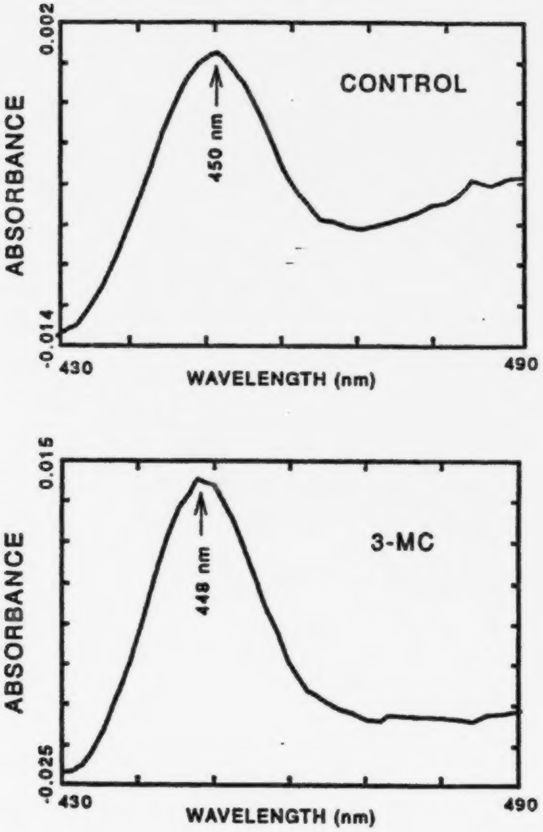


Figure 2. Absorption Spectra of Cytochrome P-450 in Control and 3-MC-Treated Rainbow Trout.

Table 1. Effects of Differential Inhibitors on BPH Activity

Additions	BPH (% of no addition)	
	Control	3-MC
None	100.0	100.0
α-NF	45.3	9.5
Metyrapone	73.8	102.3
Acetone	100.0	113.0

Discussion

Mixed function oxidase (MFO) enzymes are hemoproteins containing isoforms of cytochrome P-450 as terminal oxidases. These enzymes are critical in the metabolism of relatively nonpolar organic compounds to more polar (water soluble) metabolites. Often many xenobiotics present in aquatic environments (e.g. polyaromatic hydrocarbons, PAHs; polychlorinated biphenyls, PCBs) cause potent induction of MFO in fish (Payne et al. 1987). Results presented in this study also show that in rainbow trout the induction of MFO activities (BPH, ECOD) and P-450 level and form (P-448) was caused chiefly by a PAH-type inducer (e.g. 3-MC). This was made evident by (i) a shift of P-450 spectra from 450 nm to 448 nm, (ii) a greater induction of a P-448-linked BPH activity as compared to ECOD and (iii) specific inhibition of BPH activity by α-NF in microsomes of 3-MC treated fish.

These results thus provide (i) a good validation of technical and procedural aspects reported in this and other species and (ii) a rapid and valuable approach to assess the isoform of P-450 by employing specific inhibitors during the enzyme analysis. It is expected that future application of these and other biochemical indicators (Khan et al. 1992), can provide more powerful and sensitive tools for impact monitoring of xenobiotic chemicals.

Acknowledgements

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MULTI-SPECIES TOXICITY ASSESSMENT OF SEDIMENTS FROM THE ST. CLAIR RIVER USING *Hyalella azteca*, *Daphnia magna*, and MICROTOX (*Photobacterium phosphoreum*) AS TEST ORGANISMS.

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ABSTRACT

Industrial development and activity has occurred along the Ontario side of the St. Clair River for over 100 years. This activity significantly increased during and immediately following the second World War. Currently there are 15 major industrial facilities including petrochemical, organic and inorganic complexes discharging directly or indirectly to the river. Historically sediments near the Ontario side of the river have been highly contaminated by industrial discharges. A series of benthic macroinvertebrate studies were conducted from 1957 to 1985 to assess the area of impact. The 1957 to 1985 monitoring has shown that the zone of impact has decreased from 45 km to 12 km in length. This 12 km length corresponds to the most heavily industrialized zone. To determine the impact that sediment quality has on the benthic macroinvertebrates and water quality, and to establish baseline toxicity data, a multi-species assessment was conducted on sediments. Nine sites were sampled representing sediments located above, within and below the impacted zone. Testing procedures incorporated both whole sediment and elutriate preparations; and three test species, *Hyalella azteca*, *Daphnia magna* and *Photobacterium phosphoreum*. Results of these assessments showed little or no acute toxicity for all species tested.

BACKGROUND

The contamination of sediments along the Canadian shoreline in the St. Clair River has been a topic of concern for many years. The St. Clair River acts as the upper corridor for flow of water from Lake Huron to Lake St. Clair, the Detroit River and Lake Erie. In 1986 there were 52 known point discharges to the river from both the Ontario and Michigan sides. Dischargers included non-point sources such as urban and agricultural runoff and point source including thermal electric generating stations, municipal wastewater treatment plants, and industrial facilities consisting of organic chemicals, inorganic chemicals, petroleum refining, pulp and paper, and food processing. Total flow from point sources facilities represents approximately 20 percent of the river's daily flow. Of this 97 percent is industrial once-through or non-contact cooling water (OMOE and MDNR, 1991).

Bottom surveys of sediments have revealed that the most contaminated portion of the river is along the most industrialized portion of the river on the Ontario side (OMOE and MDNR, 1991). A large number of petrochemical related facilities, constructed beginning near the turn of the century and during and immediately following the 1940's war effort, are located along this portion of the river. Periodic collections of sediment from specific areas along the Ontario portion of the river have exceeded the Ontario Ministry of the Environment's (OMOE) guideline for open water disposal for specific parameters (OMOE and MDNR, 1991).

Typically, biological sediment quality in the St. Clair River has been assessed with macroinvertebrate benthic studies. Comprehensive macroinvertebrate benthic studies have been conducted in 1957, 1959, 1963, 1968, 1977, and 1985. (Beak 1958, 1959 and 1963; OMOE 1979; Griffith, 1989). The initial macroinvertebrate benthic study in 1957 showed a narrow zone of degradation restricted to the Canadian shoreline extending approximately 45 km downstream from Sarnia's industrialized areas. The surveys from 1957 through to 1977 indicate a reduction in the length of this zone of degradation to 25 km. Between 1977 and 1985 the zone of degradation reduced in length by 10-15 km (Environment Ontario and Environment Canada, 1986). By 1985 the zone of degradation was 12 km, suggesting that water quality and sediment conditions of the whole river had improved (Moran, 1989). Figure 1 summarizes the results of these surveys. The improvements in benthic community conditions have been attributed to the reduction in discharges of toxic materials through effluent treatment technologies and natural regeneration of river sediments.

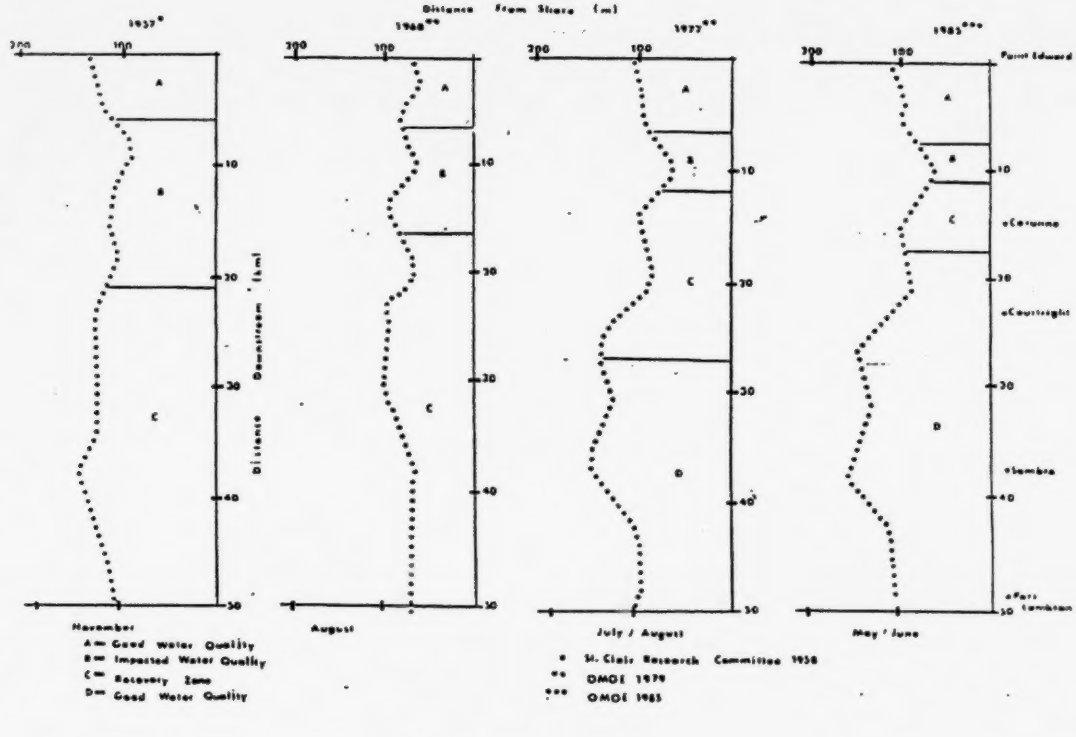


Figure 1. Summary of the Benthic Macroinvertebrate Results for the St. Clair River from 1957 to 1985 along the Canadian Shoreline (Modified from Moran, 1989)

Benthic surveys are a widely accepted technique for water and sediment quality evaluations but have a number of limitations. First, unless the chemical and physical characteristics of sediments from different locations are similar and sources of colonizing individuals are similar, it is difficult to demonstrate if any differences among populations of benthic organisms are due to the effects of water-borne pollutants or toxic substances associated with the sediments. A benthic survey alone does not quantify the degree of contamination of the sediment. Residual contaminants found within the sediments can affect benthic macroinvertebrate populations long after the source of the contamination has been stopped. Therefore, the presence or absence of benthic invertebrates alone may not accurately assess the source of toxicity providing a poor indicator of water/sediment quality.

This survey of sediment toxicity was commissioned by the Lambton Industrial Society to complement the findings of the traditional benthic studies and to provide a baseline of toxicity data for future reference as to the "state" of the St. Clair River sediments with which the effectiveness of remediation programs and/or effects of environmental mishaps can be compared. Recognizing that no single test organism can be used to adequately monitor the quality of a sediment, this study used a multi-species approach to sediment toxicity assessment.

METHODS

Sampling Locations and Procedures

A description of the sampling locations is provided in Table 1. With the exception of station #1, each sample location 2 sites were sampled; A) the shore and B) the channel bank. For station #1 three sampling locations were selected to provide information of sediments found upstream of the industrialized areas. Samples were collected from stations 4 through 7 on September 22, 1990. The remaining samples for stations 1 through 3 were collected October 4, 1990. Sediment samples were collected from the top 3 cm of the sediment surface using a ponar dredge. At each sample location, 3 to 5 random samples were collected and a composite was made. All samples were then prepared and evaluated for toxicity by the methods described in subsequent sections and submitted for particle size analysis. At each station appropriate field collection forms were prepared. This information includes the job number, person collecting sample, sample number, method of collection, surface area of sample, date, water depth, aquatic vegetation present, substrate type and consistency and any qualitative observation (example: chemical smell, presence of oil, etc.).

Table 1. Summary of Sampling Locations

STATION CODE	LOCATION
SCR 1A (Control)	Lower Lake Huron, mouth of St. Clair River
SCR 1B (Control)	South of entrance to St. Clair River not subjected to urban and industrial discharges
SCR 1C (Control)	South of Sarnia Bay and subject to runoff from the City of Sarnia and upstream of Imperial Oil Limited
SCR 2A & B	Property line between Polysar Rubber Corporation and Dow Chemical
SCR 3A & B	Property line between Suncor and Chippewa Indian reserve
SCR 4A & B	Between Ethyl Canada and Du Pont Canada outfalls
SCR 5A & B	Between Novacor Chemicals (Canada) Ltd and Corunna STP outfall
SCR 6A & B	South of Baby Creek discharge, potential for industrial runoff
SCR 7A & B	South of Stokes Point, adjacent to 11th Concession of Sombra.

Sediment Toxicity Methods

Three organisms of different habitats and behaviours were chosen to assess the quality of the sediments. These were *Hyalella azteca*, *Daphnia magna* and *Photobacterium phosphoreum* (Microtox system). The selection of three types of organisms was to provide a broad spectrum of toxicity assessment.

Hyalella azteca, an amphipod, is a common bottom dwelling crustacean which is indigenous to but not abundant in areas of the St. Clair River (Griffith, 1989). *Daphnia magna* is also a crustacean indigenous to the St. Clair River, but is free swimming rather than sediment dwelling. The use of these two species resulted in a sediment test that evaluates both an organism in direct contact with the sediment (*Hyalella azteca*) and living above the sediments (*Daphnia magna*). A second test conducted using *Daphnia magna*, used an elutriate preparation of the sample. The Microtox bioassay was also conducted using the elutriate preparation. This preparation simulates the re-suspension of sediments through such activities as dredging or ship traffic.

Amphipoda (*Hyalella azteca*) Whole Sediment Test Methods

The test organisms were obtained from the wild and then acclimatized to laboratory conditions before being introduced to the test vessels. Native populations of *Hyalella azteca* were obtained from the Speed River in Guelph, Ontario. Attempts were made to find suitable numbers of *Hyalella azteca* in the St. Clair River and associated tributaries and though present insufficient numbers could be obtained for these tests. Amphipods were separated from the sediments and plants by washing them over a series of stacked screens. The amphipods were than segregated by size with the assistance of a 1 mm pore screen. The larger juveniles required for the test procedure were removed using a glass pipette.

The test organisms were maintained for one week at laboratory conditions before being introduced into the test conditions. Holding chambers were kept at 20 C on a 16 hour light /8 hour dark cycle. Culture/dilution water used in holding and test vessels was dechlorinated tap water adjusted to a total hardness of 200 mg/L by the addition of $CaSO_4$, $MgCl_2$, and $NaHCO_3$ in order to maintain uniform water quality during the acclimatization and testing periods. Soaked maple leaves were added to the holding vessels to serve as both substrate and nutritional source to the amphipods during the acclimatization period.

Testing procedures for *Hyalella azteca* were adapted from Nebeker et al. 1984, 1986 and 1989). A 100 ml sample of packed sediment was transferred to a 500 ml jar; 400 ml of dilution water was then poured gradually down the side of the jar, to avoid overly disturbing the sediment. The test jars were aerated gently for 30 minutes prior to the start of testing and continuously throughout the duration of the test. Five *Hyalella* were transferred into each test jar using a glass pipette. Water temperature, conductivity, pH and dissolved oxygen concentration were measured at the start and completion of the test period in all test jars. At the end of the ten day test period, mortality was determined by pouring the contents of the test vessels over a fine mesh and washing away the sediment with water from a wash bottle; live and dead amphipods were counted and removed from the surface of the screen.

Daphnia magna Whole Sediment and Elutriate Test Methods

The methods for both whole sediment and elutriate tests were adapted from Nebeker et al., (1984). Both tests were 48 hour static bioassays for acute lethality. Test organisms, less than 24 hours old neonates, were obtained from a culture maintained in the laboratory.

The *Daphnia magna* whole sediment tests were conducted in 500 ml jars containing 100 ml of test sediment and 400 ml of dilution water in each of 3 replicate jars. This testing apparatus was identical to that used in the *Hyalella azteca* whole sediment tests. Test jars were left overnight unaerated to allow the sediment to settle and give more time for sediment-water contact. The water in the jars was aerated without disturbing sediment for 30 minutes prior to adding 10 test neonate daphnids to each jar. *Daphnia* were exposed for 48 hours after which mortality was determined.

Elutriates were prepared by adding 200 ml of sediment and 800 ml of dilution water to a 1 L jar which was then capped and shaken vigorously for 45 minutes. The sediment was allowed to resettle overnight before decanting the supernatant. To eliminate clay and silt particles from the elutriate, the supernatant was centrifuged for 30 minutes at 1000 g, then filtered with suction. The resulting elutriate was then used for a LC_{50}, bioassay using 3 neonate *Daphnia* in each of four replicates, 50 ml test vessels. In both tests chemical parameters were measured before the start of the test and at its termination. Dissolved oxygen concentration, pH, conductivity and water hardness were determined to ensure that the condition of the water was satisfactory for survival of the test animals.

Microtox Elutriate Test Method

A strain of the marine bacterium *Photobacterium phosphoreum* was used in this test to determine the toxicity of liquid samples. This bacterium emits light as the result of normal metabolic processes. The light emitted is measured with a photodetection device. Reduction of light over a five minute period is taken as a measure of toxicity.

The methods used were adapted from those provided by Microbics Corporation, London, Ontario and Environment Canada (Environment Canada, 1990). The test organisms were from a standardized culture of *Photobacterium phosphoreum*. A freeze dried culture of a genetically uniform strain of bacteria was obtained from Microbics. The bacteria were brought back to an active, living state by adding a liquid reconstituting solution and adjusting the temperature to a suitable level.

Since the bacterium is a marine organism, the test medium must always be maintained at 2% salinity by adding a salinity adjusting solution to the test sample. The basis of the test is the measurement of light emission from the bacteria. Liquid samples were prepared as elutriate samples as described in the previous section. For each test sample four dilutions were tested by Microtox analysis. When no toxicity was observed at any of the dilutions then the EC_{50} was reported to be greater than 100% of the sample concentration. Because of the dilution factor introduced by adding the reconstituting and salinity adjusting solutions, the maximum concentration that can be analyzed is 90% and therefore the EC_{50} should be reported as no greater than 90%.

For all sediment bioassays, appropriate quality assurance/quality control (QA/QC) procedures were observed. A stringent QA/QC plan was maintained in the bioassay laboratory including both positive controls (reference toxicants) and negative controls (clean sediments).

RESULTS

Daphnia magna Toxicity Results

For both *Daphnia magna* tests, ie., whole sediment and elutriate, no mortality or loss of mobility observed.

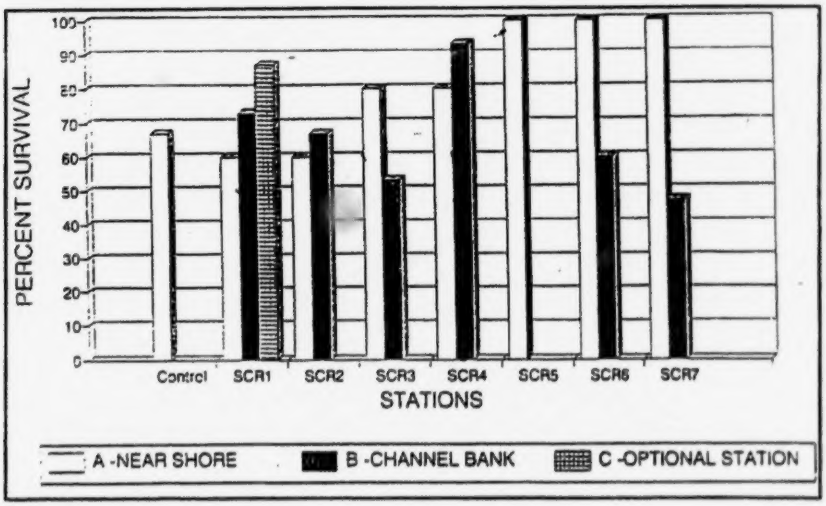
Hyalella azteca Toxicity Results

The results of the *Hyalella azteca* whole sediment test are depicted in Figure 2. The mortality data is highly variable. Because one of the control tests (dilution water) showed an average mortality of 33%, the evidence of toxicity is inconclusive. It should be noted that no bioassay showed 100% mortality and that only one of the samples had mortality greater then 50%, sample #7B with 53% mortality. For sample #5B difficulties occurred at the end of the test period in sample handling. Results for this sample have not been included in this assessment.

Microtox Toxicity Results

All Microtox assays reported an EC_{50}, greater than 90% of the sample concentration, the maximum tested concentration. Software supplied by the manufacture of the Microtox system reports the EC_{50}, as greater than 100% but for reasons discussed in the methods section the maximum concentration tested was actually 90%.

Figure 2. Summary of *Hyalella azteca* Bioassay Results



Results of sample 2A reported an EC_{50} of 78.85%. However, considering that the calculated confidence interval calculated was 0.2 to 28824.43% this sample may be considered as having no measurable toxicity with an EC_{50} greater than 90%. Further testing of sediments from this area would confirm the presence of toxicity.

DISCUSSION

Little information is available regarding the toxicity of St. Clair River sediments through the use of control laboratory bioassays. In November, 1985 sediment samples from the St. Clair River were collected by OMOE for toxicity assessment. The organisms used in sediment bioassays were *Hexagenia limbata* and *Hyalella azteca* (both pollution sensitive benthic organisms), and the Fathead Minnow, *Pimephales promelas*. At the time there was toxicity observed in samples collected adjacent to the most industrialized area of the river.

These samples were collected in the vicinity between station #2 and #3 of this survey. The amphipod, *Hyalella azteca*, was particularly sensitive to the sediments tested (Environment Ontario and Environment Canada, 1986).

To evaluate the toxicity of the sediments, this study utilized two methods to investigate two different states of the sediment. The whole sediment test models the toxicity of the sediment as undisturbed in the river bottom, whereas the elutriate test models conditions produced when contaminants are released into the water, for example when dredging.

In this investigation two crustacean species and a microbial species were used as test organisms. *Daphnia magna* was used to test the toxicity of the water above whole sediments and within elutriate water in 48 hour bioassays. The results clearly demonstrated that there was no toxicity to *Daphnia* during the test.

The second crustacean, *Hyalella azteca* was used in the whole sediment test to assess the toxicity within the sediment. There was mortality observed in the whole sediment test for *Hyalella*, however, the evidence of toxicity was inconclusive since one of the control tests (dilution water) showed 33% mortality. In the sediment bioassays conducted on samples collected November 1985, as noted above, using this amphipod, the results showed very high mortality (100%) in tested sediments while Fathead Minnows and *Hexagenia* showed much lower mortality, 0-20%, in the same sample. The observed mortality for amphipods, in the control and test sediments, may be attributed to the physical composition of the sediments rather than the "toxicity." Dewitt et al., (1988) have shown that mean amphipod survival in fine, uncontaminated, field sediments can be significantly lower than survival in more coarse sediment.

The sediments tested in this study were extremely varied in their composition. Recent concerns raised by Spies, (1989) over the influence of fine grained sediments and high total organic carbon levels on the survival of animals used in some sediment toxicity tests, should be considered when interpreting amphipod sediment toxicity data. We must conclude therefore, that the mortality observed in this bioassay cannot be attributed directly to toxicity of the sediment.

The Microtox five minute assay did not show any toxicity up to 90% of sample concentration. Microtox assays conducted on Detroit River sediments were conducted using pore water instead of an elutriate water to extract possible contaminants (Giesy et al., 1988a, 1988b). The use of pore water from St. Clair River sediments may give different results in the Microtox test because of the different sample preparation techniques. In the Detroit River sediment evaluation the Microtox assay was the most sensitive compared to *Daphnia magna* and *Chironomus tentans* assays. However, it was concluded (Giesy et al., 1988a) that the *Daphnia magna* lethality test could be used to predict highly toxic sediments in which benthic larval insect species would not be expelled to be present. It was further concluded that the Microtox assay gave sufficiently different information and it should be included only in a battery of sediment toxicity tests to accurately assess the toxicity of sediments (Giesy et al., 1988a).

The sensitivity of the Microtox test is considered to be varied when compared to *Daphnia* acute lethality tests (E.V.S. 1989). Microtox may be significantly more or less sensitive than a given multicellular organism to any particular toxic substance. Therefore, it has been recommended that micro-organisms should be included in toxicity evaluations only in conjunction with multicellular organisms to give a multi-species toxicity assessment (Environment Canada, 1990).

CONCLUSIONS

The following points summarize the main conclusions of this study:

- Whole sediment and elutriate tests showed no toxicity to *Daphnia magna*;
- Mortality was observed in the whole sediment test with *Hyalella azteca*, however, evidence of toxicity was inconclusive and may be more related to the physical composition of the sediment than its chemical content (Spies, 1989). It can be noted that no sediment sample exhibited complete mortality (100%) to *Hyalella azteca*; and
- Elutriate samples elicited no toxic response in the Microtox assay.

ACKNOWLEDGMENT

The present study was commissioned by the Lambton Industrial Society (L.I.S.) a non-profit, industry-funded, environmental cooperative consisting of 15 member companies operating in the Sarnia/Lambton area. The LIS has monitored the environment in the Sarnia and surrounding area for the past 40 years through programs to assess the quality of ambient air, water, soil, and groundwater. The authors would also like to express our appreciation to Kara Sparks (Getty) and Marianne Lines from Pollutech Environmental Limited and Scott Munro, general manager of the L.I.S. for their review of this transcript.

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INTERGRAVEL FINE SEDIMENT SAMPLER.

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ABSTRACT

Clastic stream sediments typical of riffle areas and spawning gravels are not amenable to sampling by most traditional softer sediment techniques. These sections of streams are highly productive and it is important to ensure their continued quality with respect to contaminants such as heavy metals. A stainless steel syringe sampler has been used in synoptic surveys to document baseline conditions prior to the development of mine properties. The sampler has also been used to demonstrate the sediment quality triad approach in an assessment of the biological impacts of metal contamination in a small salmonid stream. The importance of assessing metal contamination in terms other than absolute concentration is demonstrated and could be an important consideration in the development of sediment quality guidelines. Limitations and difficulties associated with the sampler are also discussed.

INTRODUCTION

Clastic stream sediments typical of salmonid stream riffle and spawning areas are difficult to sample with traditional sediment sampler techniques. As such, these areas are often overlooked in environmental effects monitoring programs to measure changes in background quality or impact assessments to evaluate the potential toxicity of contaminated stream sediments.

A stainless steel intergravel fine sediment sampler modeled after a sampler used for dissolved oxygen measurements (Ryan, 1972) has been used in synoptic surveys to assess metal levels in a number of British Columbia streams (Derksen, 1985; Derksen, 1986). The sampler has been modified further in an effort to make it "user friendly" and included in the sampler design are a number of features that allow at the time of sampling, the collection of a number of variables considered necessary to help separate the potential additive effects of different stressors such as elevated metals and depressed dissolved oxygen levels.

Sinmax Creek which discharges into Skwaam Bay of Adams Lake was chosen as an area to demonstrate the sediment triad approach (Chapman, 1990) using the intergravel fine sediment sampler. The sediment triad incorporates chemistry to measure contaminant concentrations, laboratory bioassays to measure toxicity and an in situ biological assessment of benthic invertebrate community structure. Certain areas of Sinmax Creek have been demonstrated to be contaminated with a mixture of metals including cadmium, lead, mercury, silver and zinc (Derksen, 1990).

STUDY AREA

Two sites were selected on Sinmax Creek based on the results of an earlier study (Figure 1). Sinmax Creek was sampled in May, 1990 during freshet conditions and again in

August, 1990 during low summer flows. Station SX2 was located downstream of Homestake Creek the source of metal contaminated sediments.

MATERIALS AND METHODS

The stainless steel syringe sampler is described in Schematic 1. The syringe is pushed into the streambed to the desired depth, generally 8 cm to 10 cm, and a sample composed of a mixture of intragravel water and intergravel fine sediments is withdrawn. The sample is then evacuated into the appropriate sample containers. Handling of samples from collection through to bioassay and chemical analyses is described in Figure 2.

The bioassay containers were kept cool in the field and stored in a cold room at 4°C over the weekend before decanting. In total, four days elapsed before the samples were decanted. Dissolved oxygen measurements were made at several points in the general area; benthic invertebrates were collected using a YSI model-50 dissolved oxygen meter. The diameter of the syringe barrel was sized specifically for a YSI probe. The 1 mm hole diameter syringe tip was used for dissolved oxygen samples and the 2 mm hole diameter syringe tip was used for sediments samples.

A qualitative sample for sediment particle size analysis was collected with a 10.2 cm diameter aluminum pipe, pushed 10 cm to 15 cm into the substrate. The area surrounding the pipe was excavated and a plastic plate was quickly placed over the end of the pipe before it was withdrawn. Particle size distribution was determined by standard sieve methods employed at the Water Survey of Canada sediment laboratory in New Westminster.

Six benthic invertebrate samples were collected at each site in August using a 0.093 m^2 modified Hess circular sampler with a 220 µm Nitex cloth. Aquatic insects were identified to the order level (Mounce, 1973). Stream velocity was determined with a March McBirney current meter at several points within the sample area.

All water samples collected in the field or during the laboratory bioassay period were collected in clean bottles and analyzed at the Environment Canada Laboratory in North Vancouver (Environment Canada, 1989). Sediment samples for metal analysis including sequential extraction, were analyzed by methods employed at the Environment Canada Laboratory.

After the containers had settled for several days in the cold room, elutriate samples for bioassay purposes were obtained from the decant water (intragravel water). *Daphnia magna* acute and chronic bioassays and Microtax assays were performed using methods employed by the B.C. Environment Laboratory in North Vancouver (Anon., 1990(a)(b)). Bioassay elutriate samples were analyzed for total metal content several times over the bioassay period. Dissolved oxygen and pH levels were maintained at satisfactory levels throughout the bioassay period.

The potential for metal contamination from three different 0-rings was assessed in the laboratory by drawing up deionized water through the syringe and evacuating it into clean acid-washed sample bottles.

RESULTS AND DISCUSSION

Sinmax Creek is characterized by high water hardness (>185 mg/L as CaCO), high alkalinity (>178 mg/L as $CaCO_3$) and high pH (>8.2) (Table 1). The physical features of the two sites were similar although site SX2 had an ~50% lower current velocity and ~50% lower intragravel dissolved oxygen level in August (Table 2). The sediment particle size analysis indicated that the two sites had a similar composition (Figure 3).

Intergravel fine sediment silver, cadmium, copper, lead and zinc concentrations at site SX2 were approximately an order of magnitude greater than at site SX1 (Table 3). Sequential extractions demonstrated that the metals at site SX2 were not associated with the "operationally defined" exchangeable form (Figures 4 to 7).

The bioassay results on the elutriate did not reflect any toxicity (Table 4). The mean number of offspring produced was greater for site SX2 than for the laboratory control but lower than the upstream site SX1 (Figure 8). The total metal content of the elutriate samples collected at the same time as the *Daphnia* beaker water was exchanged indicated a potential exposure to levels quite a bit higher than water quality criteria adjusted for hardness (Table 4).

The benthic invertebrate community structure at SX2, at least at the ordinal level, was not distinguishable from that of site SX1 (Figures 9 and 10). Ephemeropterans as a group are generally considered to be sensitive to a number of environmental stressors including reduced pH, elevated metals and elevated suspended solids (Derksen, 1986).

A comparison of the potential contamination from the selection of 0-rings available for the sampler indicated that the Teflon 0-rings had the least potential for contamination and that the silicon 0-rings actually used in the study had the highest potential (Table 5). The Teflon 0-rings were extremely difficult to put on and once they were compressed did not maintain a seal and had to be replaced in the field with the silicon 0-rings.

The stainless steel syringe sampler provides a means to collect a variety of environmental variables important in the assessment of the quality of the highly productive riffle and spawning areas of salmonid streams. The variables include intragravel dissolved oxygen and a mixture of in situ intragravel water and intergravel fine sediments that can be used for chemical analysis and laboratory invertebrate bioassays.

The sediment triad components of this study demonstrated that although the sediments at site SX2 were contaminated with a variety of metals, sequential extractions indicated that the metals were not in an "exchangeable" form and did not appear to exert any toxic effect as demonstrated by acute and chronic *Daphnia* bioassays and benthic invertebrate community structure. The high hardness and pH of Sinmax Creek is also likely a modifying factor. The lower intragravel dissolved oxygen levels at SX2 did not appear to be a factor effecting invertebrate community structure.

Field experience with the sampler has demonstrated that on occasion, it is difficult to draw up a sample and evacuate it. These situations occur in areas with a high proportion of fines similar in diameter to the tip hole openings. These problems although frustrating at times, can largely be managed with experience. The tip is removable and samples can be poured out rather than extruded directly. A tip that could be inserted into the syringe barrel and is sealed with 0-rings, rather than one which is screwed on, would improve the time it takes to fill the sample containers.

The Teflon 0-ring provides the least potential for contamination but once compressed, a seal cannot be maintained and it is difficult to draw up a sample. The silicon 0-ring had the highest potential for contamination. This is not considered to be critical with respect to sediment quality analyses but has to be considered a limitation with respect to using intragravel water for bioassays in low hardness streams. A sample of laboratory bioassay diluent water drawn up through the sampler could be used as a positive control and partially resolve this problem. The Buta-N 0-rings may be a compromise. Further testing on a variety of 0-rings is required at this stage.

The potential exists to use the intergravel fine sediments for a variety of sediment contact bioassays (amphipod and chironomid) and the intragravel water could be used to assess toxicity potential to salmonId eggs or alevin.

ACKNOWLEDGEMENTS

Thanks are extended to G. Mitchell for field support, the staff of Environment Canada for analytical support, and S. Lundvall for invertebrate identifications. Special thanks go to J. Donkersly and G. Van Aggelen for fitting the bioassays into their busy schedules.

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Figure 1. Sinmax and Homestake Creek Sample Sites - 1990

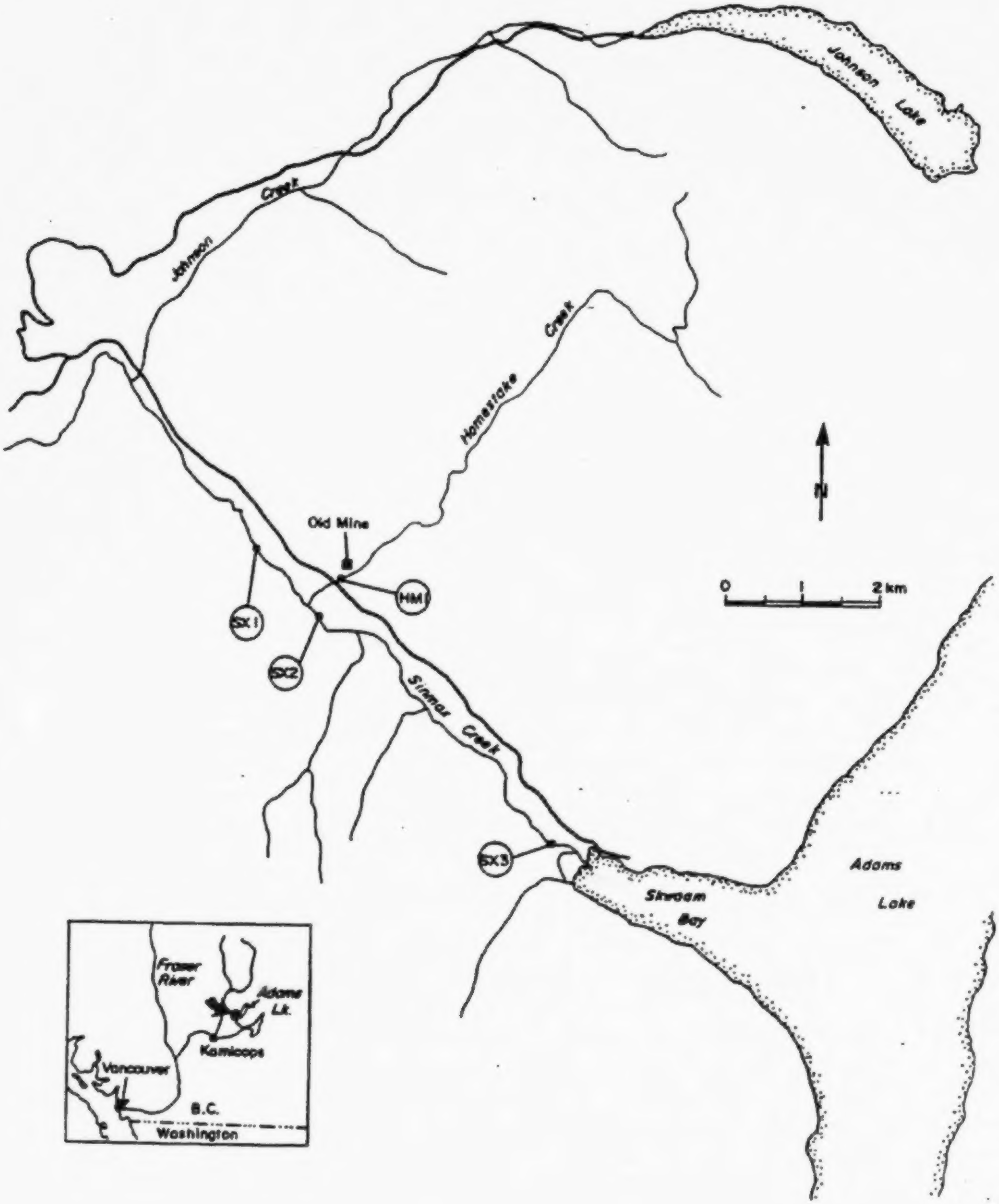
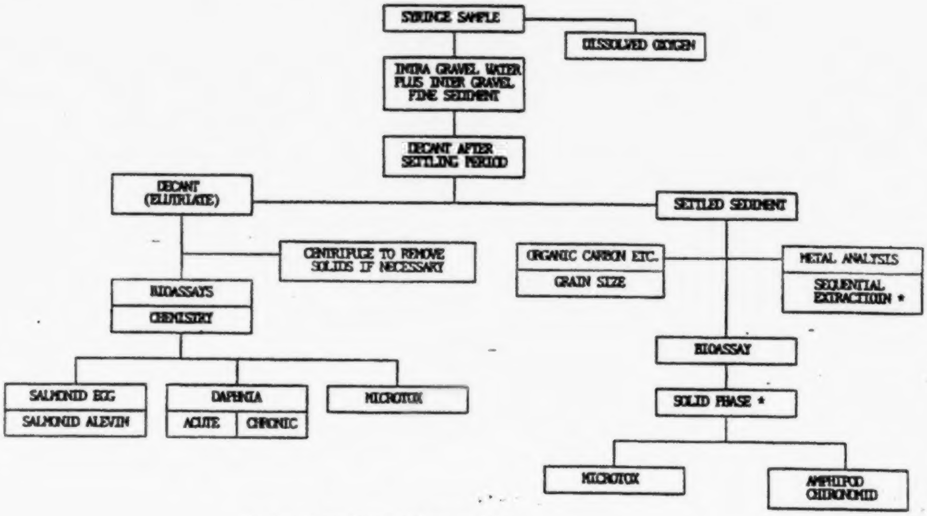
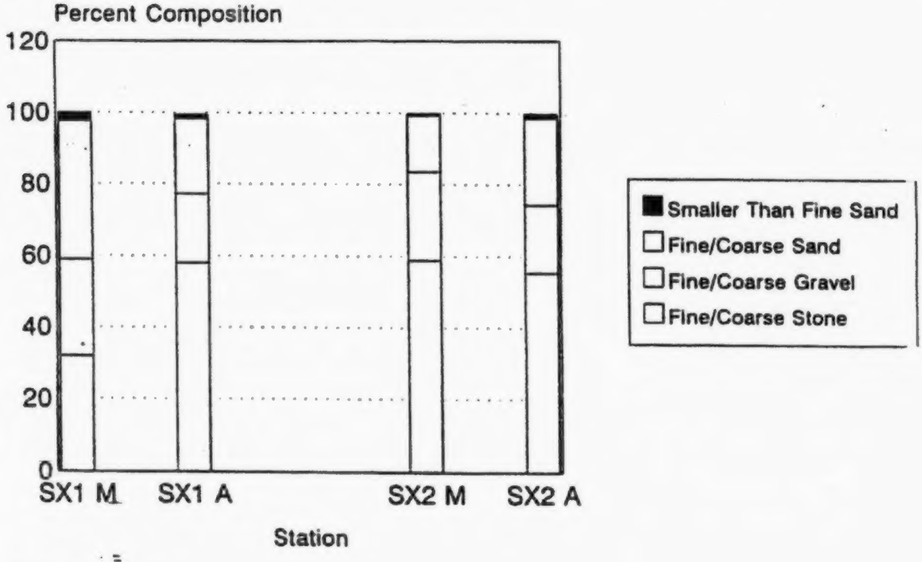

Johnson Lake
Johnson Creek
Homestake Creek
N
Old Mine
HM1
SX1
SX2
0
1
2 km
Sinmax Creek
SX3
Skwaam Bay
Adams Lake
Fraser River
Adams Lk.
Kamloops
Vancouver
B.C.
Washington

Figure 2. Sample Handling Matrix


SYRINGE SAMPLE
DISSOLVED OXYGEN
INTRA GRAVEL WATER PLUS INTER GRAVEL FINE SEDIMENT
DECANT AFTER SETTLING PERIOD
DECANT (ELUTRIATE)
SETTLED SEDIMENT
CENTRIFUGE TO REMOVE SOLIDS IF NECESSARY
ORGANIC CARBON ETC.
GRAIN SIZE
METAL ANALYSIS
SEQUENTIAL EXTRACTION *
BIOASSAYS
CHEMISTRY
BIOASSAY
SOLID PHASE *
SALMONID EGG
SALMONID ALEVIN
DAPHNIA
ACUTE
CHRONIC
MICROTOX
MICROTOX
AMPHIPOD CHIRONOMID

* Ideally samples should not be frozen

Figure 3. Stream Sediment Particle Size Analysis - Sinmax Creek



M = May 1990
A = August 1990

Figure 4. Sinmax Creek 1990 Sequential Extraction - Cadmium

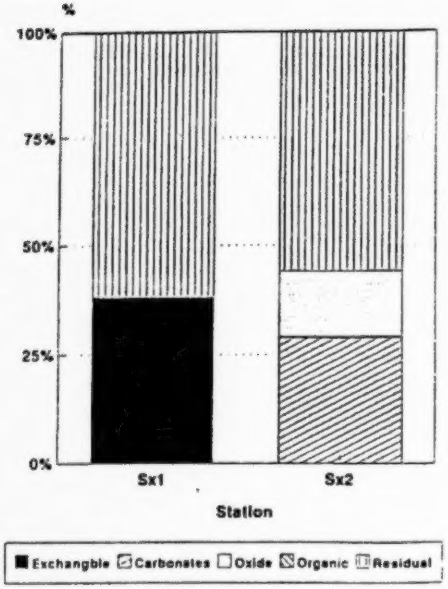


Figure 5. Sinmax Creek 1990 Sequential Extraction - Copper

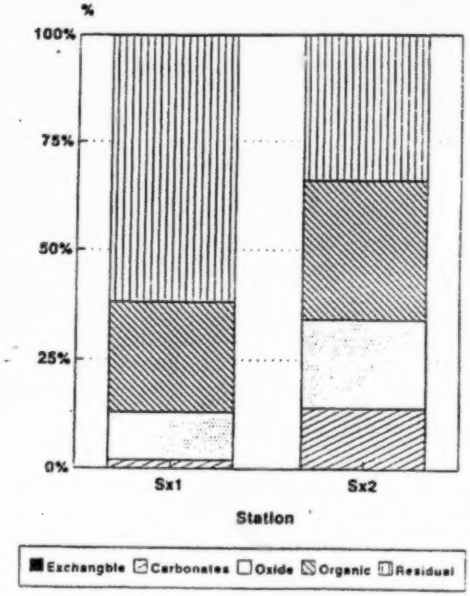


Figure 6. Sinmax Creek 1990 Sequential Extraction - Lead

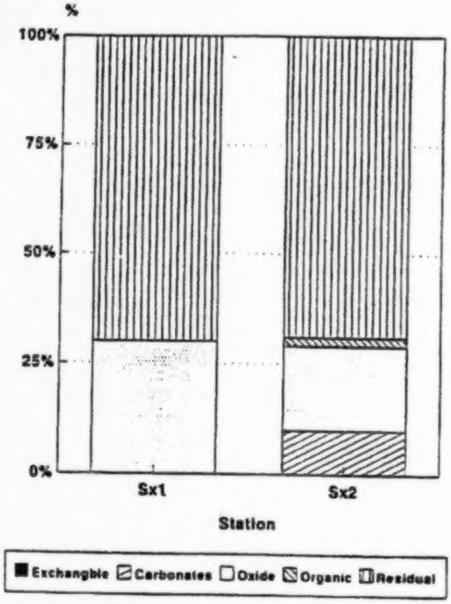


Figure 7. Sinmax Creek 1990 Sequential Extraction - Zinc

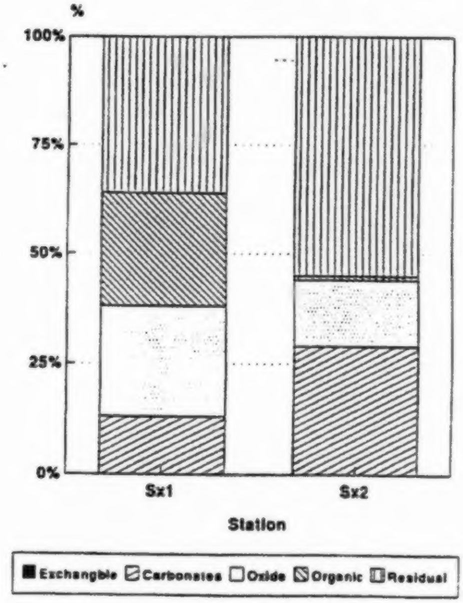


Figure 8. Daphnia Chronic Bioassay Results - May 1990 Survey

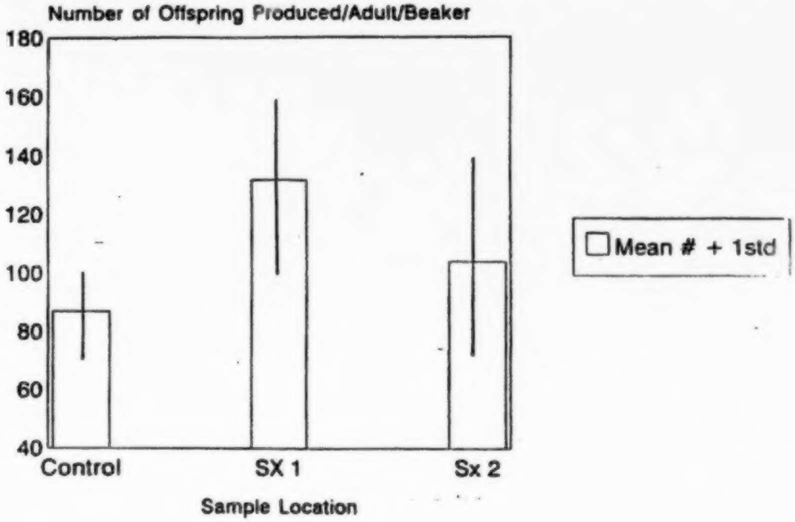


Figure 9. Benthic Invertebrates Sinmax Creek - August 1990

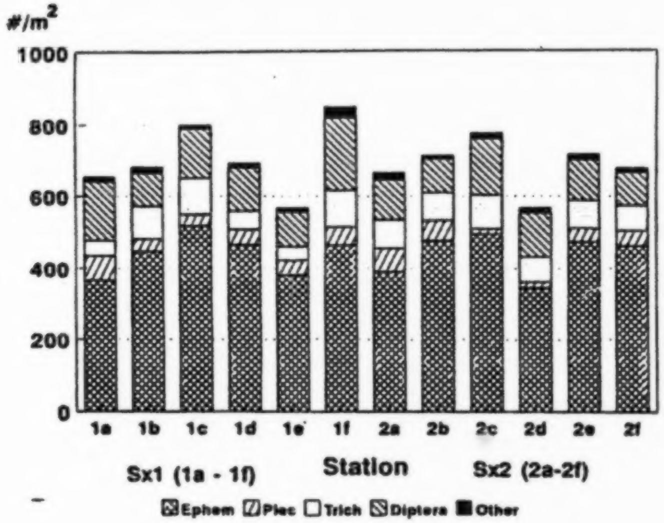


Figure 10. **Sinmax Creek Ephemeroptera Abundance**

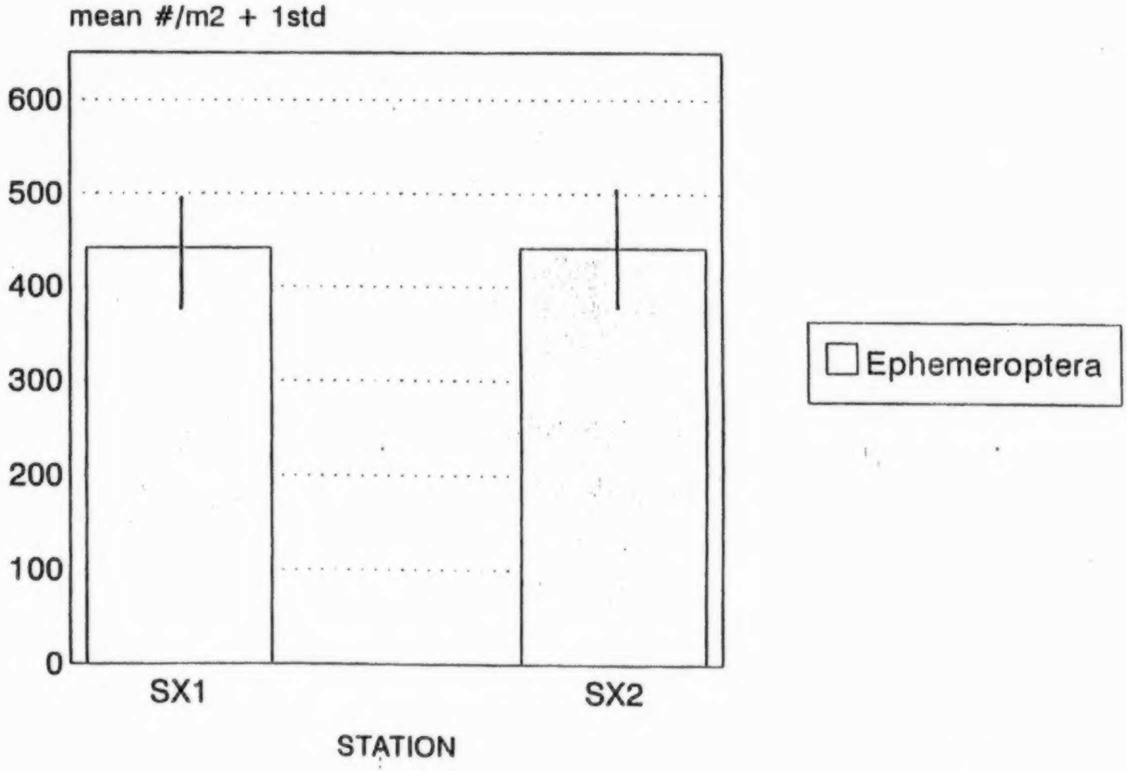


Table 1. Sinmax Creek Water Quality

Variable (mg/L)	SX1 MAY/90	SX1 AUGUST/90	SX2 MAY/90	SX2 AUGUST/90
T Alk. (as CaCO3)	178	221	-	222
T Hard. (as CaCO3)	185	236	-	241
Chloride	1.3	1.4	-	1.4
NFR	45	<7	-	<7
SO4	18.2	23.1	-	21.1
pH (units)	8.2	8.5	-	8.4

Table 2. Sinmax Creek Physical Features

STATION	SX1 - MAY/90	SX1 - AUGUST/90	SX2 - MAY/90	SX2 - AUGUST/90
SURFACE VELOCITY	140	120	100	70
DEPTH (cm)	48	23	48	21
TEMP (C)	11	13.3	10	13.2
DISSOLVED OXYGEN (mg/L) Surface	–	10.7	–	9.7
DISSOLVED OXYGEN (mg/L) Intragravel	–	10	–	4-6

Table 3. Sinmax Creek Intergravel Fine Sediment Metal Levels

STATION	SX1 - MAY/90	SX1 - AUGUST/90	SX2 - MAY/90	SX2 - AUGUST/90
VARIABLE				
Ag (ug/g dry wt.)	<2	<2	37	22
Al (ug/g dry wt.)	11600	16400	8340	11200
Cd (ug/g dry wt.)	<0.5	0.5	18	6.7
Cu (ug/g dry wt.)	22.8	43.1	348	204
Hg (ug/g dry wt.)	0.82	0.86	4.4	2.4
Pb (ug/g dry wt.)	26	23	1140	669
Zn (ug/g dry wt.)	107	148	2810	1640
SVR (%)	-	6.4	-	4.8

Table 4. Acute and Chronic Bioassays and Microtox

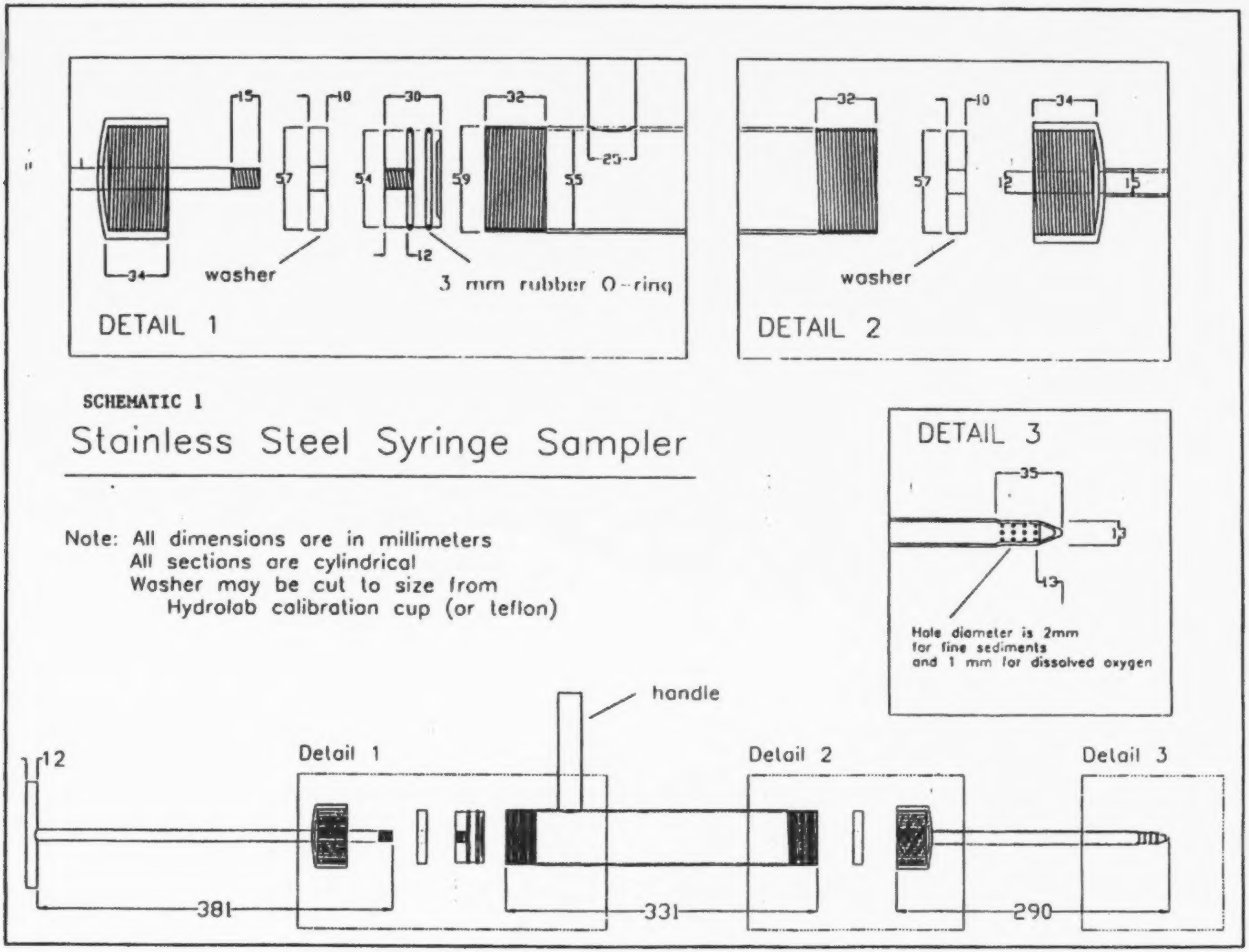
STATION and Date							
May/90	Acute Daphnia	T Ag (ug/L)	T Cd (ug/L)	T Cu (ug/L)	T Pb (ug/L)	T Zn (ug/L)	Microtox
SX1	non-toxic	<0.6	<0.1	3.3-3.9	2.9	3-8	no effect
SX2	non-toxic	4.6-4.8	0.3-0.4	18-20	40-46	110-114	no effect
	Chronic Daphnia						
SX1	non-toxic	<0.6-0.7	<0.1	5.5-5.4	9-9.5	4-18	-
SX2	non-toxic	6.9-7.0	0.7	45-46	150-172	285-290	-
August/90	Acute Daphnia						
SX1	non-toxic	-	<0.1	3.8-4.3	5.9-7.2	9-12	-
SX2	non-toxic	-	<0.1-0.3	3.8-5.9	9-27.8	49-65	-
Water Quality Criteria @ 200mg/L hardness		Ag (1)* 0.1 (13)**	Cd (1)* 1.8	Cu (2)* 8 (20.8)	Pb (3)* 11 (197)	Zn (1)* 30	

References:

(1)* CCREM, 1987
(2)* Singleton, 1987. 30-d average. (Cu) = max. not to be exceeded at any time.
(3)* Nagpal, 1987. 30-d average. (Pb) = max not to be exceeded at any time.
(Ag)** Calculated from USEPA formula for total recoverable silver which takes hardness into consideration

Table 5. O-Ring Metal Contamination

	Deionized Water Blank	TEFLON	SILICON	BUTA-N	Deionized Water Blank
TCd (ug/L)	<0.1	<0.1-0.4	<0.1-0.1	0.1-0.2	<0.1-0.3
TCu (ug/L)	<0.6	<0.6	1.0-4.8	<0.6-2.6	<0.6
TPb (ug/l)	<0.6	<0.6	<0.6-4.0	<0.6***	<0.6-0.7
TZn (ug/L)	<2	<2	<2	<2	<2-4
THg (ng/L)	<5	<5-6	6-10	6	-


15
10
30
32
25
57
54
59
55
34
12
washer
3 mm rubber O-ring
DETAIL 1
32
10
34
57
12
15
washer
DETAIL 2
SCHEMATIC 1
Stainless Steel Syringe Sampler
Note: All dimensions are in millimeters
All sections are cylindrical
Washer may be cut to size from
Hydrolab calibration cup (or teflon)
DETAIL 3
35
13
13
Hole diameter is 2mm
for fine sediments
and 1 mm for dissolved oxygen
handle
12
Detail 1
Detail 2
Detail 3
381
331
290

Schematic 1. Sinmax and Homestake Creek - Sample Sites 1990

THE EFFECTS OF PCB 126 ON HEPATIC MIXED FUNCTION OXYGENASE ACTIVITY AND STEROIDOGENIC CAPACITY OF THE GOLDFISH *(CARASSIUS AURATUS)*.

[1]Karen Kidd, [2]Glen Van Der Kraak and [3]Kelly Munkittrick [1]Department of Zoology, University of AB, Edmonton, AB; [2]Department of Zoology, University of Guelph, Guelph, ON; [3]Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, ON.

ABSTRACT

Experiments were conducted to determine the effects of PCB 126 on liver mixed function oxygenase (MFO) activity and testicular steroidogenesis in male goldfish (*Carassius auratus*). Fish were injected intraperitoneally with corn oil containing PCB 126 at final doses of 0, 0.05 or 0.5 ug/g. Hepatic MFO activity (ethoxyresorufin-o-deethylase; EROD) and the plasma steroids testosterone and 11-ketotestosterone were measured 4, 8 and 16 days after injection. Goldfish were injected with Ovaprim (gonadotropin releasing hormone, 0.1 ug/g and domperidone, 10 ug/g) on days 4, 8 and 16 to stimulate gonadotropin synthesis in the pituitary and subsequent steroid production in the testes. Ovaprim-stimulated testosterone and 11-ketotestosterone levels were measured 24 hours after injection. Results of these experiments indicate that PCB 126 increases EROD activity in the treated fish up to 15-fold over the controls and depresses circulating testosterone and 11-ketotestosterone levels.

INTRODUCTION

Increased hepatic mixed function oxygenase (MFO) activity and depressed circulating sex steroids have been a consistent finding in several species of fish found downstream of pulp mills. The relationship between the two phenomenon is unclear at this time although steroid depression is known to be due in part to decreased gonadal synthesis and a reduced sensitivity of the gonad to gonadotropin (Van Der Kraak *et al.*, 1992). Attempts to examine the relationship have been hampered by an inability to replicate steroidal depression in lab fish exposed to bleached kraft mill effluent (BKME). We were interested in using a model compound to duplicate the biochemical lesions under controlled laboratory conditions. Coplanar 3,3',4,4',5-pentachlorobiphenyl (PCB 126) was chosen for this study due to its high concentrations in fish from the Great Lakes (Smith *et al.*, 1990) and its potential for disrupting steroid function. Laboratory experiments have found that fish exposed to commercial PCB mixtures exhibit increased hepatic MFO activity and decreased plasma sex steroids (Melancon and Lech, 1983; Thomas, 1988).

The objective of this study was to determine the effects of PCB 126 on MFO activity and circulating sex steroid levels in the male goldfish (*Carassius auratus*). Ethoxyresorufin-o-deethylase (EROD) activity, and basal and [4]Ovaprim-stimulated testosterone and 11-ketotestosterone levels were measured in fish injected with corn oil containing PCB 126 at final doses of 0, 0.05 and 0.5 ug/g.

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[4] Ovaprim is a combination of gonadotropin (Gth) releasing hormone and domperidone and is used to stimulate gonadotropin secretion by the pituitary and subsequent steroid production by the gonads.

METHODS

Male goldfish (27.5 ± 6g) were acclimated for 7 days in 25 l experimental tanks. Tank water was dechlorinated, aerated and maintained at 15°C. Fish were fed once a day to satiation and were held under a constant photoperiod (14 h D:10 h L). All sampling was done between 0900 and 1100.

Experiment 1: On day 0, 2 groups of 18 fish were injected intraperitoneally (ip) with corn oil containing PCB 126 (0 and 0.05 ug/g). On days 4, 8 and 16, 6 fish per group were anesthetized using trizane methane suphonate (MS222) and bled via caudal puncture. Three fish per group were bled and sacrificed, and the livers were removed. The remaining fish per group were injected with Ovaprim (gonadotropin releasing hormone, GnRH, 0.1 ug/g; domperidone, 10 ug/g) and bled and sampled after 24 hours.

Experiment 2: Three groups of 8 fish were injected ip with corn oil containing PCB 126 (0, 0.05 and 0.5 ug/g). After 4 days, fish were anesthetized (MS222), bled and injected with Ovaprim (GnRH, 0.1 ug/g; domperidone, 10 ug/g). All fish were bled after 24 hours and sacrificed, and the livers excised.

Mixed Function Oxygenase Analysis

After removal, livers were immediately frozen in liquid nitrogen. Samples were thawed on ice, homogenized in buffer (0.5% KCl) and EROD activity was measured using the substrate ethoxyresorufin as described in McMaster *et al.* (1992).

Steroid Analysis

All blood samples were kept on ice and then centrifuged. Plasma was ether-extracted and analyzed for testosterone and 11-ketotestosterone using the radioimmunoassay procedures described by Van Der Kraak (1984) and Wade and Van Der Kraak (1990).

Statistical Analysis

The nonparametric Wilcoxon-Mann-Whitney test was used to determine if significant differences exist between PCB-treated and control fish ($p \leq 0.05$, * indicates significance).

RESULTS

EROD activity was elevated significantly in PCB-injected fish (0.05 ug/g) after 4 days and further increased up to 15-fold over activity in control fish (day 16/17, Figure 1).

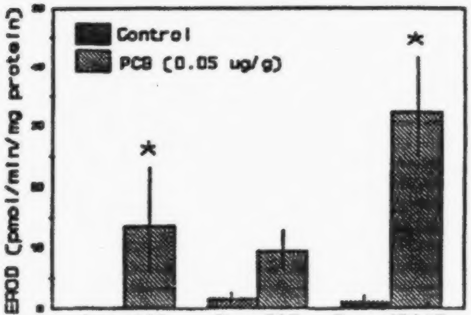

Figure 1. EROD activity in the goldfish 4, 8 and 16 days following injection with corn oil containing PCB 126 (0 or 0.05 ug/g fish)(mean ± S.E., n=7 or 8; samples pooled on day 4/5, 8/9, and 16/17).

PCB 126 (0.05 ug/g) reduced plasma levels of testosterone and 11-ketotestosterone after 4 and 8 days respectively. Testosterone levels in fish injected with Ovaprim were significantly lower after days 9 and 17 while 11-ketotestosterone concentrations were depressed on day 9 (Figure 2).

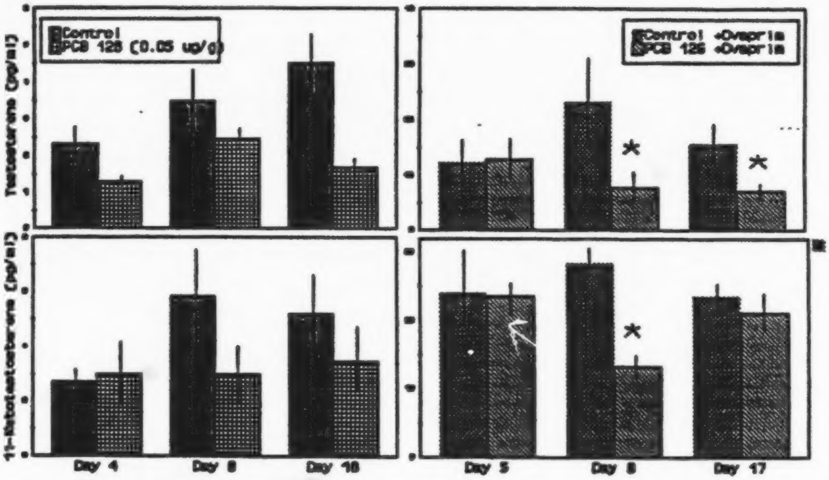

Figure 2. Plasma testosterone and 11-ketotestosterone levels in goldfish 4, 8 and 16 days following injection with corn oil containing PCB 126 (0 or 0.05 ug/g fish) and on days 5, 9 and 17, 24 hours after injection with Ovaprim (0.5 ul/g fish) (mean ± S.E., n=3-6).

EROD activity was significantly elevated in fish 4 and 5 days following treatment with PCB 126 (0.05 and 0.5 ug/g)(Figure 3).

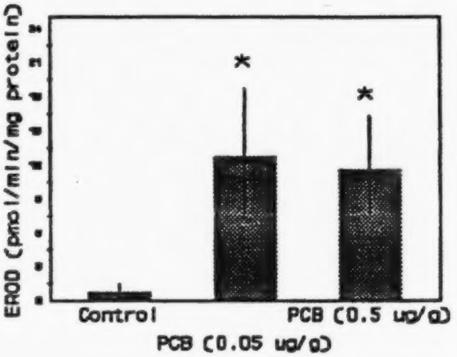

Figure 3. EROD activity in the goldfish on days 4 and 5 following injection with corn oil containing PCB 126 (0, 0.05 or 0.5 ug/g fish)(mean ± S.E., n=7 or 8).

Fish injected with PCB 126 (0.05 ug/g) had significantly reduced circulating 11-ketotestosterone levels when compared to the controls. Levels of both testosterone and 11-ketotestosterone are reduced in PCB-treated fish (0.05 and 0.5 ug/g), 24 hours after injection with Ovaprim (Figure 4).

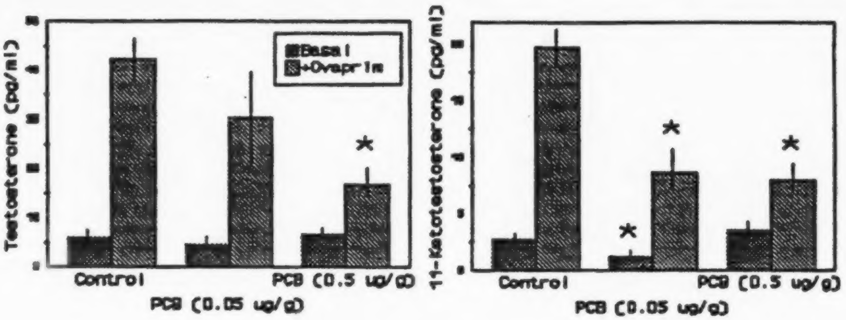

Figure 4. Plasma testosterone and 11-ketotestosterone levels in goldfish on day 4 following injection with corn oil containing PCB 126 (0, 0.05 and 0.5 ug/g) and on day 5, 24 hours after injection with Ovaprim (0.5 ul/g fish)(mean ± S.E., n=3-8).

DISCUSSION

In both experiments, goldfish treated with PCB 126 exhibited increased hepatic MFO activity when compared to the controls. These results are consistent with other studies of fish exposed to PCB 126 and PCB 77 (Janz and Metcalfe, 1991), and BKME (McMaster *et al.* 1991; Munkittrick *et al.* 1991).

The reduction in circulating testosterone and 11-ketotestosterone levels in PCB-treated fish may be due to a lesion at the level of the pituitary and/or gonad. Recent studies by Van Der Kraak *et al.* (1992) have found that bleached kraft mill effluent depressed steroid levels in fish through a reduction in gonadotropin (Gth) production, an impairment of gonadal responses to Gth and a difference in peripheral metabolism of the steroids. Thomas (1988) found that Arochlor 1254 reduced plasma steroid levels in fish through a decrease in Gth secretion by the pituitary. Rats exposed to 2,3,7,8-TCDD, a stereoisomer of PCB 126, exhibited reduced Gth secretion by the pituitary (Bookstaff, 1990a,b) and depressed steroidogenesis due to a second block at the level of the gonad (Kleeman *et al.*, 1990; Moore *et al.*, 1991).

CONCLUSION

The results of these experiments indicate that PCB 126 induces EROD activity and depresses circulating steroid levels in the male goldfish. Further experiments need to be conducted to determine if PCB 126 decreases steroid levels directly at the level of the pituitary and/or gonad or indirectly through a difference in peripheral metabolism.

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Moore, R. *et al.* (1991) 2,3,7,8-TCDD inhibits steroidogenesis in the rat testis by inhibiting the mobilization of cholesterol to cytochrome P450. Toxicol. Appl. Pharmacol. 109:85.

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DEVELOPING A QUALITY ASSURANCE/QUALITY CONTROL PROGRAM FOR A TOXICITY TESTING LABORATORY.

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ABSTRACT

Laboratories must comply with increasingly rigorous requirements to assure the acceptability of data generated in toxicity tests. Establishment of formal quality assurance/quality control (QA/QC) programs is becoming an essential component in order to ensure the quality of data generated, its reproducibility and scientific defensibility. The effort required to implement a QA/QC program will vary with laboratory size and the requirements of pertinent regulatory agencies. Major items to consider in developing a program are preparation of a master QA/QC plan (usually in the form of a QA/QC Manual), establishment of standard laboratory operating procedures, training of personnel, standardization of techniques, establishing equipment maintenance records, formalizing reporting structure with respect to responsibility, establishing regular reference toxicant testing programs and maintaining control charts, and, setting out policies for judging whether data meet acceptability criteria or not. Staff must be trained to maintain an acceptable level of detail in written recordkeeping; their input during program development will facilitate ease of implementation. In addition to reviewing all data and maintaining records, an independent QA/QC unit should undertake random internal audits to ensure the program is maintained once it has been implemented.

DEFINITIONS

Quality Assurance (QA): The total integrated program organized and designed to provide accurate and precise results, to assure data reliability. Included are selection of proper technical methods or laboratory procedures; sample collection and handling; data quality objectives; evaluation of data; quality control; and, qualifications and training of personnel.

Quality Control (QC): Specific actions for obtaining prescribed standards of performance as part of a quality assurance program. Included are standardizations, calibration, replicates, and control and reference samples suitable for providing statistical estimates of data confidence.

THE QUALITY ASSURANCE PLAN

A formal, written Quality Assurance Plan forms the foundation of any QA/QC program. Typically this Plan is set out in a QA/QC Manual, to be used in the laboratory on a day-to-day basis, or to perform audits. The scope of the Plan will vary with the size of the laboratory, ranging from a few pages for a small facility to one or more volumes for a larger one. The QA/QC Manual should describe policies for the following:

- Laboratory structure/responsibilities training;
- Study management;
- Sample handling;
- Test methods;
- Performance evaluations/intercalibrations;
- Data management and acceptability;
- Reporting;
- Records/archival; and
- Audits - internal and external.

STANDARD OPERATING PROCEDURES

Written standard operating procedures (SOPS) must be developed for every task routinely performed in the laboratory. In addition to the SOPs for specific toxicity test techniques, SOPs are also required for general tasks such as receiving samples, cleaning glassware and calibrating instruments. Each SOP needs to be tailored to describe the specific method used by a particular laboratory. Manufacturer's instructions (for equipment usage) and published test methodologies (e.g. Environment Canada and USA EPA protocols) usually form the basis for an SOP. Where these documents describe options, the SOP should specify which method to follow. Detailed SOPs assist laboratory staff to identify exact equipment requirements and the expected time tasks should take. Use of SOPs ensures that procedures are performed in a consistent manner; they are an important tool in personnel training. SOPs need to be revised regularly to ensure they are up to date.

ELEMENTS OF QUALITY CONTROL

Negative Controls - All tests are conducted using well-established negative (clean) controls, such as clean diluent water (or clean diluent water and clean sediment). The test is repeated if the control does not meet the acceptability criteria for a particular test.

Positive Controls (Reference Toxicants) - All toxicity tests should include positive (toxic) controls, conducted with standard reference toxicants. Control charts should be constructed for each species and reference toxicant used.

Test Organisms - Only healthy organisms of similar size and life history stage are used for toxicity tests. All test organisms used for a batch of tests must be from the same source. Taxonomic identifications must be confirmed.

Replication - The number of replicates required varies from one test protocol to another, but should always be sufficient to account for variability in test organism response.

Blind Testing/Randomization - Test samples should be distinguished by a code to prevent laboratory personnel knowing their identity. Arrangement of treatment containers should be randomized during testing.

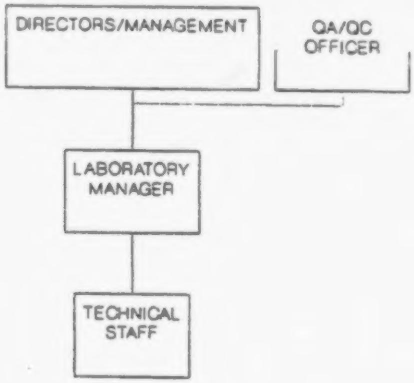
Instrument Calibration - Calibration of instruments (dissolved oxygen, pH and conductivity meters, refractometers, balances) is required to ensure that accurate measurements are made throughout a test and that equipment is operating correctly. Each instrument should be calibrated according to the manufacturer's instructions, and details recorded in a logbook.

Water Quality Measurement/Maintenance - Proper water quality conditions must be maintained to ensure survival of test organisms, and to ensure that undue stress (unrelated to the test materials) is not exerted on them. Appropriate water quality parameters must be measured at the start and end of a test as a minimum, and preferably every 24 h.

Standard Laboratory Procedures - Standard laboratory procedures should be followed in all testing. These include use of established methods, proper documentation, proper cleaning, avoidance of contamination and maintenance of appropriate test conditions. All unusual observations or deviations from established procedures should be recorded and reported.

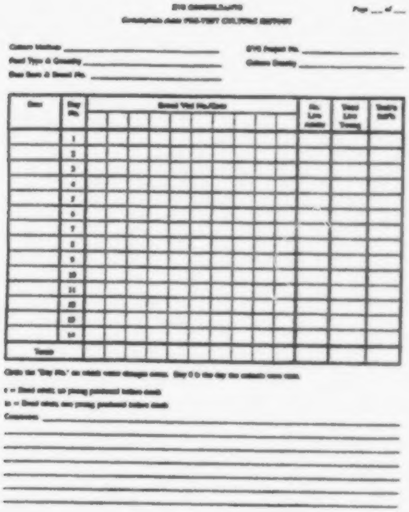
ORGANIZATION AND TRAINING

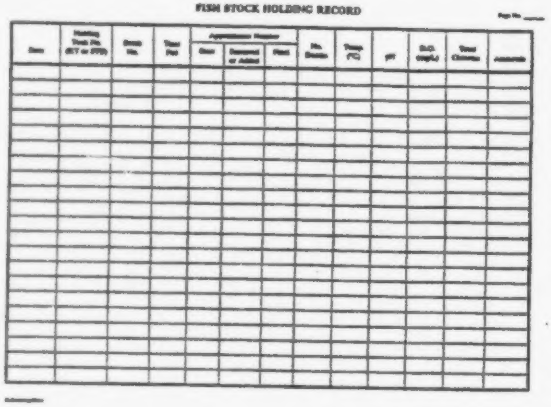
A quality assurance/quality control program also includes the laboratory structure, with respect to organization of staff and their responsibilities, and documentation of training. A simple example organizational chart is shown. A typical arrangement in a private laboratory is that there will be a Laboratory Manager who is responsible for overseeing the activities of the technical staff. This includes ensuring that tests are performed correctly and within holding time restrictions, and that equipment is functioning properly. This person will frequently act as the Project Manager or Study Director for projects involving toxicity tests. The Laboratory Manager reports to senior company management. Responsibilities for equipment management, sample receipt and archival of records should also be given to specific individuals. To provide independent oversight of data generated, a QA/QC Officer is required. This person must act independently from laboratory personnel and report directly to senior management. The QA/QC Officer is responsible for reviewing all toxicity test data prior to report generation, ensuring that all records and logbooks are maintained, updating control charts, and for performing internal audits to monitor laboratory performance. Most technical training will be on-the-job, pairing new staff with experienced personnel. Records should be maintained to document when staff have received in-house training in specific tasks and when they have demonstrated certain proficiency levels. This information may be required by regulatory agency's accreditation programs.



INSTRUMENT CALIBRATION

Calibration of instruments is required to ensure that accurate measurements are being made throughout the course of a test and to ensure the piece of equipment is operating correctly. Each water quality instrument (dissolved oxygen, pH and conductivity meters, refractometers) and analytical or toploading balances should be calibrated at the start of each day (and any time the environmental conditions are changed). Each piece of equipment should have a logbook for daily recording of calibration information, repairs, replacement, etc. Entries should be made every day the instrument is used; periods when the instrument is unavailable due to repairs, etc. should also be logged so there are no gaps of information. The calibration logbook for a particular instrument should be maintained on file for five years after the instrument is no longer in operation. Each instrument should be calibrated according to the manufacturer's instructions; these should be included in the SOPs for equipment use.



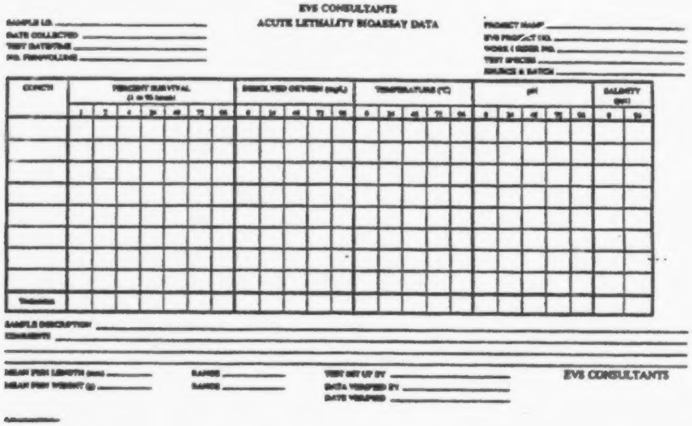


FISH STOCK HOLDING RECORD

LABORATORY RECORDS

Maintenance of complete and accurate records is an essential part of laboratory QA/QC. Records must be complete enough to allow a person unfamiliar with a particular study to reconstruct it. Written records include: the test protocol, information about sample collection, identification and handling, preparation of test solutions, sources and condition of test organisms, test initiation, monitoring and termination, statistical analysis and documentation of any unusual observations of deviations from protocol. Bound logbooks with numbered pages are the preferred method for recording information other than that on test datasheets. Other information should be stored in designated files or binders for easy reference. Examples of forms used in the laboratory are provided below.

All entries in logbooks or on datasheets must be made legibly in black, indelible ink and initialled by the person making the entry. All calculations should be initialled by the person making them and the person checking them. To make a correction, a single line is made through the incorrect entry and the new entry is initialled and dated. An explanation for the correction is also required.



EVS CONSULTANTS
ACUTE LETHALITY BIOASSAY DATA

SAMPLE I.D. ____
DATE COLLECTED ____
TEST DATE/TIME ____

PROJECT NAME ____
EVS PROJECT NO. ____
TEST SPECIES ____
SOURCE & BATCH ____

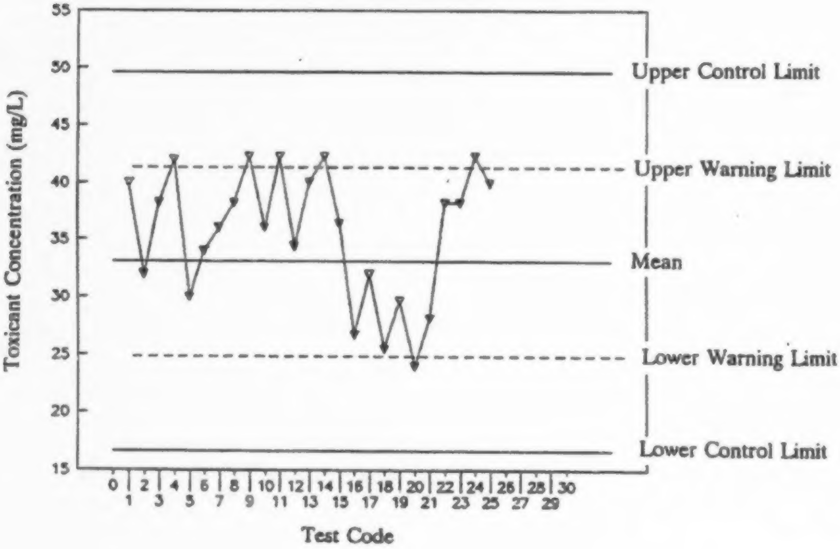
SAMPLE DESCRIPTION ____
COMMENTS ____

TEST SET UP BY ____
DATA VERIFIED BY ____
DATE VERIFIED ____

EVS CONSULTANTS

CONTROL CHARTS

Control charts should be constructed to monitor reference toxicant performance. There should be a separate chart for each reference toxicant and each species tested in the laboratory. Once a sufficient number of data points have been collected, a laboratory mean and standard deviation should be calculated. The minimum number of data points required is 5, although 20 has also been suggested. This cumulative laboratory mean value should be re-calculated with successive data points until the results are stable. Upper and lower control limits (mean ± 2SD) for test acceptability are also plotted on the chart. These limits represent 95% confidence limits for the data. Warning limits (mean ± SD) should also be included. The warning limits serve as a trigger if successive test results begin to approach the control limit. These charts should be available in the laboratory and updated with the results of each reference toxicant test. Typically, the QA/QC Officer is responsible for monitoring the data for trends in increasing or decreasing sensitivity. If the results of a reference toxicant test fall outside the control chart limits, the test procedures and health/source of the test organisms should be reviewed; subject to those findings, the test may have to be repeated.



Reference Toxicant: Sodium Dodecyl Sulphate

Test Species: Rainbow Trout

AN INVESTIGATION OF THE POTENTIAL FOR *in situ* BIOREMEDIATION OF OIL SANDS TAILINGS.

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Syncrude Canada Ltd. extracts bitumen from the oil sands formation in northeastern Alberta and upgrades the bitumen into a synthetic crude oil. Tailings water produced during bitumen extraction has been shown to be acutely toxic to aquatic organisms. Syncrude has determined that naphthenic acids are the primary source of acute toxicity within oil sand tailings waste. Naphthenic acids are naturally occurring components of crude oil and bitumen; elevated levels within oil sand tailings may be the result of the caustic hot water flotation procedure used to extract bitumen from the oil sands.

The potential for *in situ* bioremediation of oil sand tailings was investigated by determining the ability of indigenous bacteria to biodegrade naphthenic acids. A mixed bacterial culture enriched from oil sand tailings was found to be capable of growth on a commercially available naphthenic acid mixture. When sodium naphthenates (30 mg/L) were added to a minimal salts medium and inoculated with the mixed bacterial culture, gas chromatography revealed that many components of the naphthenic acid mixture were biodegraded within 8 days of incubation. The same mixed bacterial culture was also tested against the naphthenic acid fraction extracted directly from oil sand tailings. The tailings extract was diluted into the minimal salts medium in sealed flasks and inoculated with the enrichment culture. The production of carbon dioxide indicated microbial mineralization of components within the oil sands extract. Microtox analysis determine that microbial activity resulted in a reduction in acute toxicity of the tailings extract.

Introduction

A synthetic crude oil is produced from bitumen extracted from the Athabasca oil sands formation in northeastern Alberta. Bitumen is separated from the oil sands using a caustic soda hot water flotation process which requires large volumes of water and produces a tailings waste which is a mixture of water, solids (sand and clays) and non-extracted bitumen (50:50:1) (Mackinnon and Retallack 1982). Bitumen extraction by Syncrude Canada Ltd. results in the annual accumulation of 20 - 30 x 10^6 m^3 of tailings waste. All water used in bitumen extraction is contained within a tailings pond which is approximately 30 m deep and covers a surface area greater than 17 km^2.

Water from the tailings pond is acutely toxic, as indicated by a 96-h rainbow trout LD_{50} of <10% (v/v) and a Microtax EC_{50} of <30% (v/v) (Mackinnon and Boerger 1986). Chemical analysis has traced the primary source of acute toxicity to a complex group of polar organic acids which are believed to be naphthenic acids (Mackinnon and Boerger 1986). Naphthenic acids are a complex mixture of predominantly mono- and polycycloalkane carboxylic acids with aliphatic side chains of various lengths. Naphthenic acids are naturally occurring components of crude oil (Seifert 1975, Fan 1991) and bitumen, although, elevated levels within the tailings pond may be the result of the process by which bitumen is extracted (Mackinnon and Boerger 1986).

The potential for in situ bioremediation of oil sand tailings was investigated by determining the ability of indigenous microorganisms to biodegrade naphthenic acids. A mixed bacterial culture enriched from oil sand tailings was found to be capable of growth on naphthenic acids. Growth of the enrichment culture on the naphthenic acid fraction extracted directly from oil sand tailings was also examined.

Methods

Naphthenic acids-degrading enrichment culture: A product containing a mixture of naphthenic acid sodium salts (NAS) was purchased from Kodak chemicals (Rochester, NY).

The initial inoculum for the enrichment culture was a sample of oil sands tailings taken 1 m below the tailings sludge/free water interface in Syncrude's tailings pond. Naphthenic acids-degrading bacteria were enriched on a solution of 100 mg/L NAS in BHM minimal salts medium (Wyndham and Costerton 1981).

Cell number within the enrichment culture was estimated using viable plate counts (VPC). Serial dilutions were plated onto agar-hardened half-strength BHI medium and the plates were incubated for 4 d at room temperature.

Biodegradation of naphthenic acids: An inoculum of enrichment culture (1% v/v) was added to 30 mg/L of NAS in BHM medium (50 mL) within 125-mL flasks. The flasks were incubated at room temperature on a shaker, and, after 2, 4, 6, and 8 days, duplicate inoculated flasks and 1 control flask were sacrificed for GC analysis. Contents of the flasks were acidified to pH < 2 using 4 N H_2SO_4. Stearic acid (6 mg/L) was added as an internal standard, and the contents of the flask were extracted 4 times with methylene chloride. To aid in the GC analysis, carboxylic acid groups were derivatized to methyl esters.

Chemical analysis was performed using a Hewlett-Packard gas chromatograph with a flame ionization detector. The chromatograph contained a 30-m DB5 capillary column which was initially held at 90°C for 2 min and then increased, at a rate of 4°C/min, to a final temperature of 270°C. Injection port and detector temperature were both held at 250°C. The carrier gas, helium, had a linear flow rate of 45 cm/sec.

Naphthenic acid extract from oil sand tailings: The naphthenic acid fraction was extracted from oil sand tailings using the method described by Mackinnon and Retallack (1982) and Mackinnon and Boerger (1986). Briefly, tailings were acidified to pH = 2.5 in order to convert carboxylic acid groups into a form in which they bind to tailings solids. Tailings water was separated from the solids by centrifugation, and the carboxylic acids were released from tailings solids using a 0.1 N NaOH solution. The tailings extract was concentrated to a level

50 times greater than in the tailings pond by adding a volume of the NaOH solution which was less than the amount of tailings water originally removed.

Mineralization of tailings extract: Tailings extract was diluted into 15 mL of BHM within 50-mL serum vials and inoculated with the enrichment culture (1% v/v). The serum vials were sealed with rubber stoppers and incubated at room temperature on a shaker. At intervals of between 3 and 5 days, triplicate inoculated and control vials were acidified to pH <2 and samples of headspace gases (0.5 mL) were analyzed for the production of carbon dioxide. The contents of a separate set of inoculated vials and one control vial were filter sterilized (Millipore, Millex, 0.45 µm) and used for Microtox testing.

Carbon dioxide was analyzed using a Fisher gas partitioning chromatograph (model 1200). Helium was used as a carrier gas at a flow rate of 30 mL/min. The column temperature was maintained at 50°C. Peak areas were integrated on a Hewlett Packard 3390A integrator.

Microtox testing: Microtox assays were performed on a Microbics Model 500 (Carlsbad, CA) using standard methods.

Results and Discussion

Naphthenic acids are a complex mixture of cycloalkane carboxylic acids with aliphatic side chains of various lengths. The cycloalkanes are predominantly 5- or 6-carbon rings; compounds containing more than 6 rings have been reported (Fan 1991).

GC analysis of the Kodak naphthenic acid sodium salts revealed a series of many overlapping peaks, or a "hump," positioned before the internal standard, stearic acid (Figure 1). The addition of the enrichment culture resulted in the reduction in the size of the "hump" within 4 days of incubation, indicating that many components within the mixture, were biodegraded. The two remaining peaks were considered to be contaminants introduced during sample preparation. The peak closest to stearic acid was identified as a phthalate, and the other peak is palmitic acid, which may have been introduced with the internal standard, stearic acid (P. Fedorak, personal communication).

Two colony types were found to dominate the enrichment culture, and they have been identified as *Pseudomonas stutzeri* and *Alcaligenes denitrificans*.

The naphthenic acids-degrading enrichment culture was also found to be active against the naphthenic acid fraction extracted directly from oil sands tailings. The production of carbon dioxide for two dilution levels of tailings extract are shown in Figure 2. The amount of $C0_2$ produced in the inoculated vials was corrected for the level of $C0_2$ in the control vials. The results indicated mineralization of components within the tailings extract, with $C0_2$ production reaching a plateau within 9 days of incubation. Viable plate counts revealed an increase in cell number by approximately two orders of magnitude when the enrichment culture was grown on tailings extract (data not shown).

Microtox analysis was used to indicate whether microbial activity against components within the tailings extract resulted in a reduction in acute toxicity. Results revealed that, at both dilution levels, acute toxicity was reduced, although not completely alleviated, by microbial activity (Figure 3).

These results indicated that naphthenic acid-degrading bacteria can be enriched from oil sand tailings, and that these bacteria were capable of reducing the acute toxicity of the naphthenic acid fraction extracted directly from oil sands tailings. The residual toxicity of the inoculated tailings extract may be due to the presence of naphthenic acids which are highly recalcitrant to microbial degradation. Further studies will focus on the biodegradation of these compounds, and on the degradation of naphthenic acids within oil sands tailings ponds.

References

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Seifert, W.K. 1975. Carboxylic acids in petroleum and sediments. In: Progress in the Chemistry of Organic Natural Products. Springer Verlag, Berlin, Vol. 32, pp. 1-49.

Wyndham, R.C. and J.W. Costerton. 1981. Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca oil sands deposit. Appl. Environ. Microbiol. 41:783-790.

Figure 1. Biodegradation of naphthenic acids by the oil sand tailings enrichment culture as indicated by the reduction in the series of overlapping peaks associated with naphthenic acid sodium salts. The peak labelled "st" indicates the intimal standard, stearic acid.

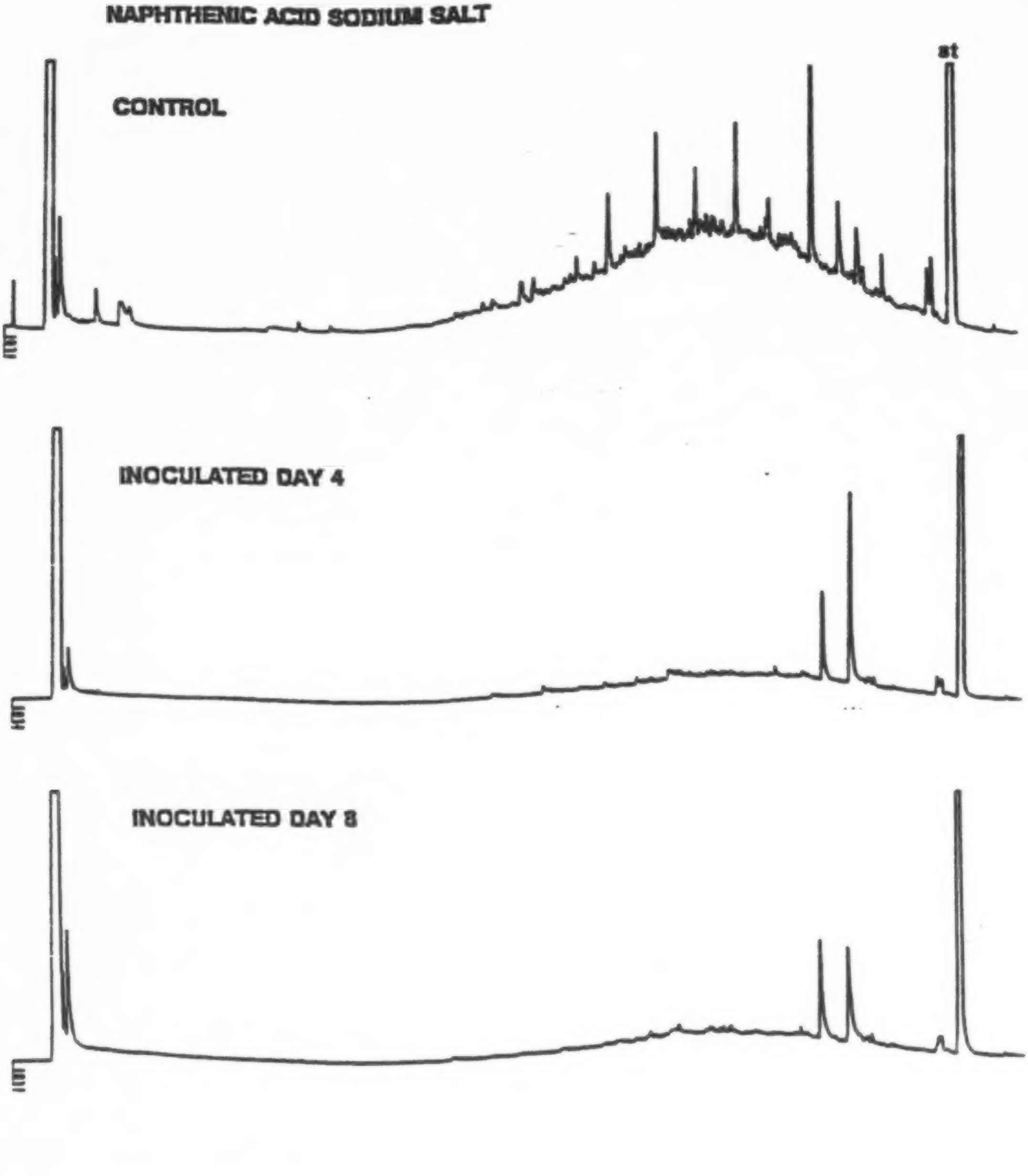


Figure 2. Mineralization of the naphthenic acid fraction of oil sand tailings. The naphthenic acid extract was diluted by either 1/20 or 1/50 into a minimal salts medium and inoculated with a mixed bacterial culture capable of degrading naphthenic acid sodium salt. Bars represent 1 standard deviation from the mean.

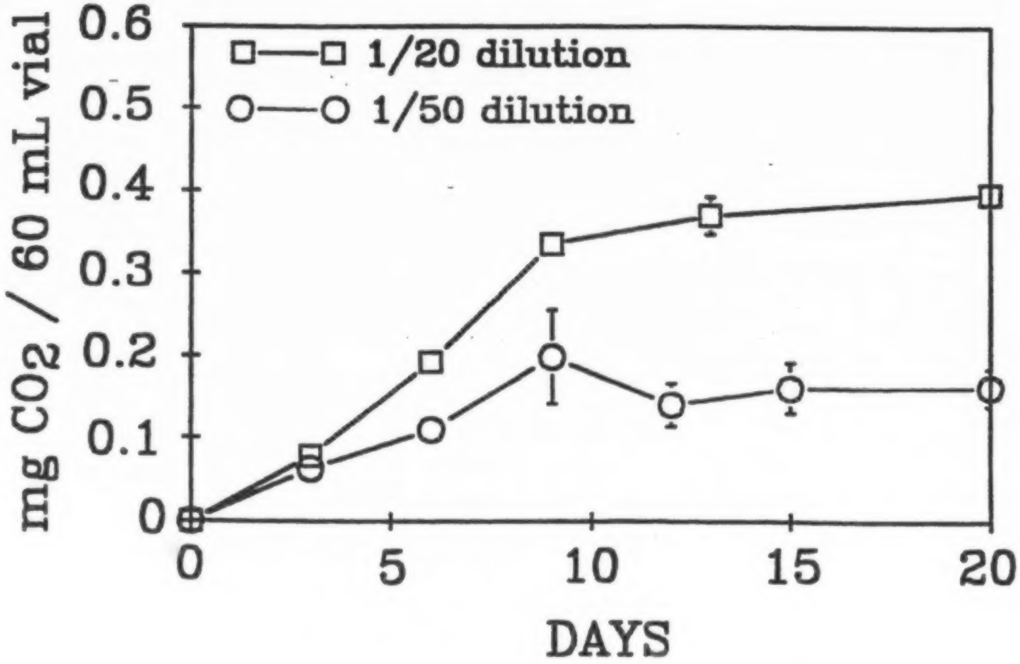
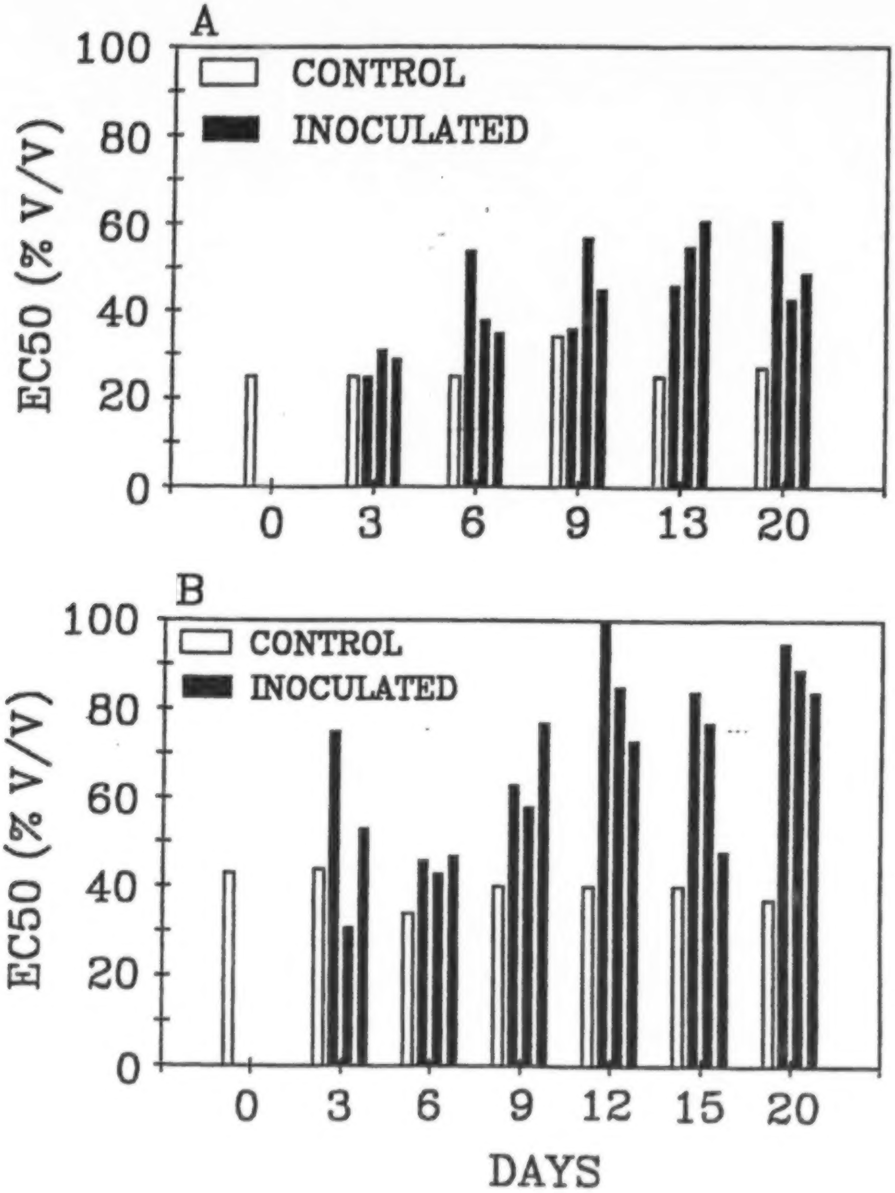


Figure 3. Microtox analysis showing the reduction in acute toxicity with biodegradation of components within the naphthenic acid fraction of oil sands tailings when diluted 1/20 (A) and 1/50 (B) into minimal salts medium. Microtox EC50 values for three inoculated vials (solid bars) and one control vial (open bar) are shown for each sampling day.



BEST STUDENT AWARD/PRIX POUR LE MEILLEURE ÉTUDIANT

Awards for the Best Student Platform and Poster Presentations are presented annually by the Workshop Organizing Committee to encourage student participation. The 1992 winners are:

Best Student Platform Presentation

Darwin M.A. Monita
University of Calgary, Calgary, Alberta

Best Student Poster Presentation

Karen Kidd
University of Alberta, Edmonton, Alberta

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The Proceedings of the Annual Aquatic Toxicity Workshops have been published as a series of technical reports listed below. Copies of recent Proceedings may be available from A. Niimi, Continuity Chairman, Aquatic Toxicity Workshop, Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6. Copies of most Proceedings are available for a charge from Micromedia Limited, 165 Hotel de Ville, Place du Portage, Hull, Quebec J8X 3X2, (819 770-9928). Their catalog numbers (MLCN) are listed below where applicable.

Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquatic. Sci.

Proceedings of the Eighteenth Annual Aquatic Toxicity Workshop: September 30-October 3, 1991, Ottawa, Ontario. Edited by A.J. Niimi and M.C. Taylor. Can. Tech. Rep. Fish. Aquat. Sci. 1863: 381 p. (MLCN: 97-6/1863).

Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, BC. Edited by P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuk and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p. (MLCN: 91-06176).

Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p. (MLCN: 90-01805).

Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p. (MLCN: 88-04587).

Proceedings of the Thirteenth Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p. (MLCN: 88-01709).

Proceedings of the Twelfth Annual Aquatic Toxicity Workshop: November 5-8, 1985, Thunder Bay, Ontario. Edited by G.W. Ozburn. Can Tech. Rep. Fish. Aquat. Sci. 1462: 229 p. (MLCN: 86-5828).

Proceedings of the Eleventh Annual Aquatic Toxicity Workshop: November 13-15, 1984, Vancouver, BC. Edited by G.H. Green and K.L. Woodward. Can. Tech. Rep. Fish. Aquat. Sci. 1480: 330 p. (MLCN: 86-1103).

Proceedings of the Tenth Annual Aquatic Toxicity Workshop: November 7-10, 1983, Halifax, Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 475 p. (MLCN: 86-1103).

Proceedings of the Ninth Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mackay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p. (MLCN: 84-3262).

Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish Aquat. Sci. 1151: 255 p. (MLCN: 83-2515).

Proceedings of the Seventh Annual Aquatic Toxicity Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert, and M. Speyer. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p. (MLCN: 82-0070).

Proceedings of the Sixth Annual Aquatic Toxicity Workshop: November 6 & 7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p. (MLCN: 81-1492).

Proceedings of the Fifth Annual Aquatic Toxicity Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann, Fish Mar. Ser. Tech. Rep. 862: 342 p. (MLCN: 80: 4061).

Proceedings of the Fourth Annual Aquatic Toxicity Workshop, November 8-10, 1977, Bayshore Inn, Vancouver, BC. Edited by J.C. Davis, G.L. Greer and I.K. Birtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p. (MLCN: 80: 4022).

Proceedings of the Third Annual Aquatic Toxicity Workshop, November 2-3, Halifax, Nova Scotia. Edited by W.R. Parker, E. Pessah, P.G. Wells and G.F. Westlake. Environ. Prot. Ser. Tech. Rep. EPS-5-AR-77-1.

Proceedings of the Second Annual Aquatic Toxicity Workshop, November 4-5, 1975, Rexdale, Ontario. Edited by G.R. Craig, Ontario Ministry of the Environment.

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